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(54) **Synthetic peptides specific for the detection of antibodies to HCV, diagnosis of HCV infection and prevention thereof as vaccines.**

(57) The present invention relates to peptides which are immunoreactive to antibodies to HCV or NANBHV and a method of detecting the presence of HCV or NANBHV antibodies in body fluids by using the peptides as the antigen. The peptides are selected from both the envelope and non-structural protein regions of the HCV or NANBHV. The detection method includes enzyme linked immunosorbent assay or other immunoassay procedures. The peptides and conjugates or polymers thereof are also useful as immunogens in generating high titer antibodies to HCV or in vaccines.

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INTRODUCTION

The present invention relates to peptides specific for the diagnosis and prevention of hepatitis C virus (HCV) infection, or non-A non-B hepatitis (NANBH). More particularly, the present invention is directed to synthetic peptides which are specific for the detection of antibodies to HCV in body fluids and immunoassays using the same. The invention also includes the use of the synthetic peptides in compositions as antigens for eliciting the production of monoclonal and polyclonal antibodies against HCV and as immunogens in vaccines for the prevention of NANBH or HCV infection.

In recent years, non-A, non-B hepatitis (NANBH) has become the most common form of post-transfusion hepatitis. Studies involving the experimental inoculation of chimpanzees provided evidence that the infectious agent was a lipid-containing virus resembling members of the Togaviridae family.

Recently, this etiological agent, termed hepatitis C virus (HCV) has been shown to be an RNA virus with a genome size of ~ 10 kilobases encoding a single polyprotein which can be further processed into several structural and nonstructural proteins (1-4). Additional computer-assisted protein analysis demonstrates that HCV shares sequence similarity with the polyproteins of animal pestiviruses and flaviviruses as well as members of two plant virus supergroups (5).

More recently, a number of reports have led to an increasingly coherent understanding of the function of various regions of the virus and of the relationships among genomic fragments isolated from variants or closely related viruses.

A summary of the HCV structure, beginning at the N terminus of the virus, follows. The HCV comprises a structural protein region and nonstructural (NS) protein regions. The structural protein region is further divided into capsid and envelop proteins. The NS protein regions are further divided into NS-1 to NS-5 regions (3).

The postulated capsid region (AA1-AA120) has been shown to contain highly immunoreactive conserved epitopes with enhanced sensitivity in the detection of hepatitis C infection (6-8). The region appears to consist of two segments of equal length (AA1-61, AA62-AA120), which are homologous to one another, perhaps as a result of a gene duplication, and are also homologous to the N terminal core region of yellow fever virus (9), also a flavivirus (Table 1A). Both halves, as represented by peptides VIIIE (AA2-AA62) and IXD (AA65-AA119), disclosed in application serial No. 558,799, have been shown to be immunoreactive. A genomic fragment of a NANBH virus cloned by Arima et al. (10), designated clone 2, contains a Gly-Pro-Arg-Leu-Gly sequence identical to residues 39-43 in peptide VIIIE (Table 1B), placing this clone 2 fragment in the putative core region of a related virus. Two other sequences from NANBH viruses, cloned by Reyes et al. (11) and by Arima et al. (clone 1) (12), show sequence similarities with the capsid region of yellow fever virus (Table 1C). Thus, there appears to be a number of related viruses, all of which have highly immunogenic capsid regions, as evidenced by the ease of cloning. Variants of hepatitis C (J, J-1, J-4) are also highly conserved in this region (2-4), so the other clones mentioned by Arima et al. may be from different viruses, rather than from variants of HCV.

Mishiro and colleagues have isolated a cDNA clone from the plasma of a chimpanzee infected with NANBHV which codes for a host cellular sequence bearing an epitope which is reactive with sera from individuals who are PCR positive for HCV (13). The sequence of the immunoreactive peptide (GOR epitope) is not encoded by HCV and was reported not to resemble a published sequence of HCV spanning three-quarters of the genome (1) or the 5'-terminal sequence of HCV (2) covering the upstream quarter of the genome. However, inspection of the GOR epitope sequence revealed 47% homology with an N-terminal fragment covered by peptide VIIIE described in UBI Applications Serial No. 558,799. Lesser degrees of homology were obtained from comparison with the N-terminus of the yellow fever virus capsid protein (33%) (9) and the protein segment corresponding to clone 1 of Arima et al. (37.5%) (12) (See Table 1D).

The presence of antibodies which are cross-reactive with the GOR epitope sequence in HCV infected individuals may be explained by structural similarity of the GOR epitope with the corresponding region of the HCV capsid protein. Compared with anti-C100, antibodies to the C100 region, previously identified by Houghton et al.; antibodies to peptide VIIIE share the following characteristics with anti-GOR: they both are present in some but not all anti-C100 positive sera; they can be detected in anti-C100 negative sera from both acute and chronic NANBH patients; they appear earlier than anti-C100 in the seroconversion series; they are detected in more seroconversion panels than anti-C100 (13); and they are present in 1-2% of normal controls and 15-20% of HBsAg positive individuals. Early NANBH assays reported to react with host-determinant cytoplasmic antigens may in fact have detected anti-HCV capsid protein cross-reactivity.

The postulated envelope (env) region consists of amino acids 120 to 400. The env glycoproteins of flaviviruses are key targets for immunization because the env region is a major antigen of free viral particles and plays a central role in flavivirus biology. The env region mediates binding to cell receptors end

probably facilitates fusion to membranes. It also induces protective immune responses after vaccination or natural infection with a flavivirus (14,15) and stimulates cell-mediated immunity (16). The type-specific epitopes on env are the ones most closely associated with protective immune responses to flaviviruses (17-19). There are e number of hypervariable regions in the HCV env region, based on a comparison of US and Japanese strains (2), which may indicate epitopes for strain specific reactivity.

The non-structural protein NS-1, in addition to the small M protein of the envelope, has been shown to contribute to protective immunity in dengue fever (20,21). Inspection of sequences and hydrophobicity profiles shows that the HCV NS-1 region contains two similar domains (Table 1E). A dominant motif in, this region is cysteine pairs separated by tive or more amino acids.

The NS-2 region is of unknown function and little has been reported on Its characteristics.

By enalogy with yellow fever virus, the HCV NS-3 region may contain protease ectivity required for viral replication (22). A trypsin-like serine protease active site has been localized in yellow tever virus by means of site-directed mutagenesis of NS-3 to a catalytic triad consisting of His-53, Asp-77 and Ser-138. The corresponding region in HCV is the N-terminal third ot NS-3, with the critical residues being His-1103, Asp-1127 and Ser-1188. The remainder of the HCV NS-3 region consists of a region which shows immunoreactivity. This region appears to consist of three subregions homologous to one another (Table 1F) and these subregions bear a distant relationship to the repeated segments of the NS-1 region.

The most widely studied region to date is the NS-4 nonstructural region. Although its function is unknown, it contains highly Immunoreactive regions, primarily in the region designated as C100 by Houghton et al. (1), which became the basis for a HCV diagnostic test using recombinant technology. A high degree of structural homology is observed between part of the C100 HCV sequence with a corresponding region in the yellow fever virus (Table 1G). While this region detects antibody to the virus primarily responsible for NANBH (23), experimentally it has been shown in prior United Biomedical Inc.'s application Serial No. 558,799 and numerous recent reports that there are shortcomings in both sensitivity and specificity in the tests relying on the C100 polypeptide as an antigen. However, synthetic peptides from the NS-4 region described in prior application Serial No. 558,799 overcome the problem of non-specific reactivity.

The nonstructural region proximal to the C terminus of HCV is NS-5, the site of polymerase (pol) ectivity. The Gly-Asp-Asp sequence in this region is conserved across many viruses(11). Maeno et al. have isolated a clone corresponding to a sequence upstream of the pol site in the NS-5 region which is immunoreactive and which reacts specifically with sera trom patients in the chronic phase of NANBH(24).

Through an extensive series of experiments involving serological validation using select specimens chosen from the screening of thousands of sera with hundreds ot caretully designed synthetic peptides, we have further characterized the capsid protein related immunoreactive peptides and have identified additional immunoreactive epitopes contained within the envelope, NS-1, NS-2, NS-3, and NS-5 protein regions.

Synthetic peptides have been increasingly used to map antigenic or immunogenic sites on the surface of proteins, an epproach recently termed "site-directed-serology". We, at United Biomedical, have taken this approach to identify and characterize highly antigenic epitopes on the envelope and core proteins of HIV and to develop sensitive and specitic immunoassays for the detection of antibodies to HIV (previously designated HTLV-III) (25-27). See U.S. Patent 4,735,896, issued April 5, 1988 and (U.S. Patent 4,879,212 issued Nov. 7, 1989, the contents of which are, hereby, fully incorporated by reference (28,29). Subsequently, a series of finely mapped and well-characterized HTLV-I/II related synthetic peptides were employed in the development of synthetic peptide-based diegnostic assays for the detection of HTLV-I/II antibodies in infected individuals (30,31). See also U.S. Patent 4,833,071 issued May 23, 1989, U.S.S.N. 07/297,635 filed January 13, 1989 and USSN 07/469,294 filed January 24, 1990. These assays have provided superior sensitivity, excellent specificity, and, in certain cases, an unmatched capability to differentiate infections between two closely related viruses, thus overcoming many of the existing problems associated with biologically-derived tests based on either viral lysates or recombinant DNA-derived proteins.

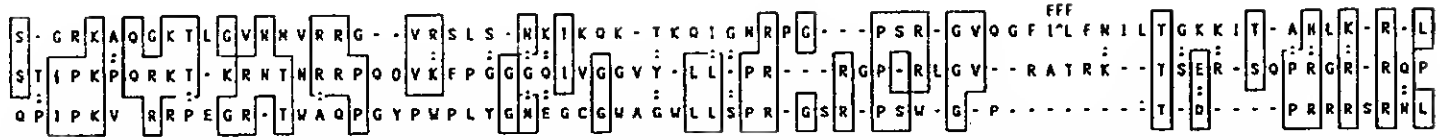
It is, theretore, en objective ot the present invention to employ the identified and characterized immunoreactive HCV peptides in the development of a detection or diagnostic procedure to identify and monitor HCV infection.

A further objective is to chemically synthesize a test reagent which can then be used to detect the presence of antibodies to HCV in body fluids and to diagnose NANBH.

Another objective is to develop a vaccine which, when introduced into healthy mammals, including humans, will stimulate production of efticacious antibodies to HCV, thereby providing protection against HCV infection.

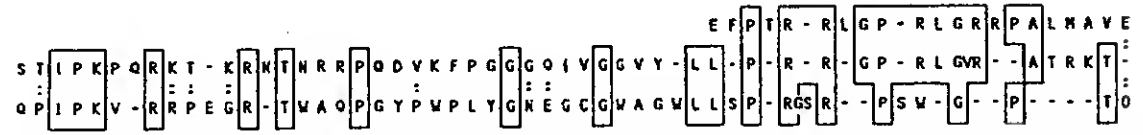
A further objective is to provide a synthetic immunogen which can be used in mammals for the development of monoclonal and polyclonal antibodies to HCV.

Table 1A



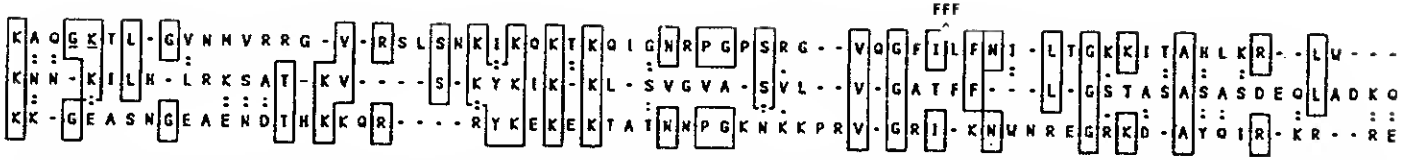
Amino acid sequences (single letter code) derived from the corresponding N-terminal capsid protein of the Yellow Fever Virus (AA2-AA68, upper line; Ref. 9) and the Hepatitis C Virus (AA2-AA64, middle line; and AA63-AA119, lower line; Ref. 2) are aligned for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.

Table 1B



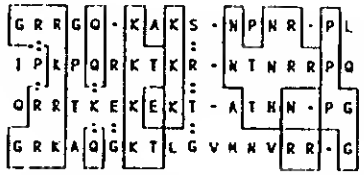
Amino acid sequence (single letter code) derived from a segment of Arima et al.'s HANBHV-protein clone 2 (upper line; Ref. 10) is aligned with segments of the N-terminal capsid protein of the Hepatitis C Virus (AA2-AA52, middle line; and AA63-AA111, lower line) for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.

Table 1C



Amino acid sequence (single letter code) derived from the N-terminal capsid protein of the Yellow Fever Virus (AA5-AA69, upper line; Ref. 9), another NANBHV sequence cloned by Reyes et al. (AA1-AA55, middle line; Fig. 3, Ref. 11) and a third NANBHV sequence cloned by Arima et al (AA5-AA66, lower line; Ref. 12) are aligned for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.

Table 1D



GOR Epitope Sequence (Ref.13)

AA4-AA19 Segment of HCV Capsid Peptide VIIIE of prior application serial no. 558,799

Arima et al. Clone 1 (AA22-AA37, Ref.12)

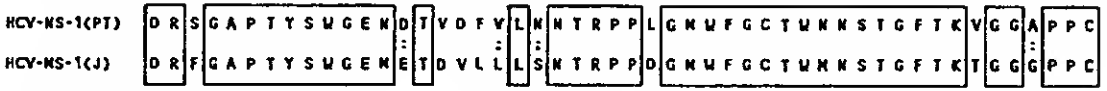
Yellow Fever Virus (AA3-AA19, Ref.9)

Amino acid sequences (single letter code) derived from the GOR Epitope (upper line; Ref.139), a segment of the HCV capsid peptide VIIIE representing HCV AA4-AA19 of prior application (second line), AA22-AA37 of the NANBHV sequence (clone 1) reported by by Arima et al (third line; Ref. 12) and a segment of the Yellow Fever Virus N-terminal capsid protein (AA2-AA19, Ref.9) are aligned for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.

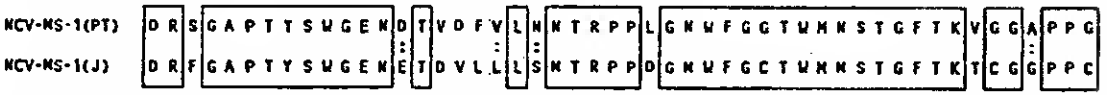
Table 1E

Amino acid sequences (single letter code) derived from two segments of the HCV NS-1 portein (upper line, AA459-AA508; and lower line, AA520-AA569; are aligned for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.

Amino acid sequences (single letter code) derived from three HCV strains (J-1, J-4 and J) for a segment of the NS-1 protein (AA459-AA508); are aligned for comparison of homology.



Amino acid sequences (single letter code) derived from two HCV strains (PT and J) for a segment of the NS-1 protein (AA520-AA569) are aligned for comparison of homology.



Amino acid sequences (single letter code) derived from two HCV strains (PT and J) for a segment of the NS-1 protein (AA520-AA569) are aligned for comparison of homology.

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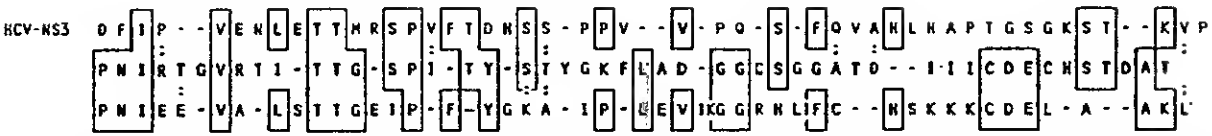
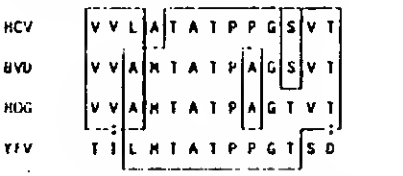
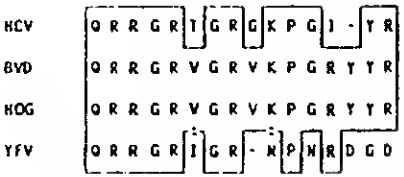
Table 1F



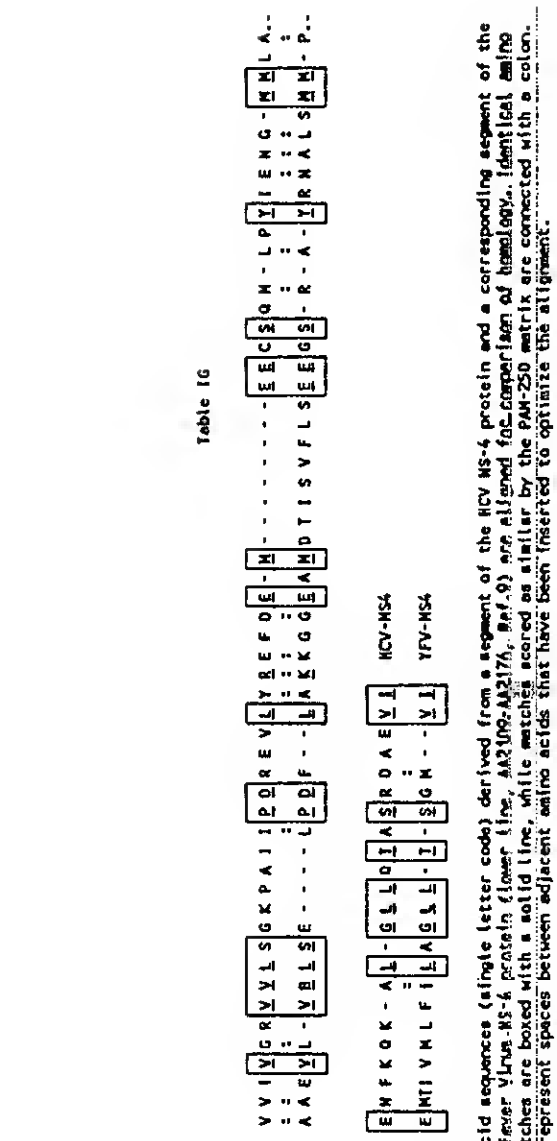
Amino acid sequences (single letter code) derived from three segments of the HCV NS-3 protein (AA1195-1241, upper line; AA1276-AA1324, middle line; and AA1360-AA1407, lower line) are aligned for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.





Multiple alignment of two highly conserved segments encoded within the NS-3 protein region (single letter code) of HCV (AA1344-AA1356, upper Table; and AA1486-AA1500, lower table respectively), Bovine Diarrhea Virus (BVD, AA2025-AA2037; AA2181-AA2196), Hog Cholera Virus (HOG, AA1886-AA1898; AA-2042-AA2057) and Yellow Fever Virus (YFV AA1800-AA1812; AA1944-AA1958) are aligned for comparison of homology.

Table IG



Amino acid sequences (single letter code) derived from a segment of the HCV NS-4 protein and a corresponding segment of the Yellow fever virus NS-4 protein (lower line, AA2109-AA2174, Ref.9) are aligned for comparison of homology. Identical amino acid matches are boxed with a solid line, while matches scored as similar by the PAM-250 matrix are connected with a colon. Dashes represent spaces between adjacent amino acids that have been inserted to optimize the alignment.

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BRIEF DESCRIPTION OF THE INVENTION

According to the present invention, a series of synthetic peptides representing immunoreactive regions

of the postulated envelope protein and nonstructural proteins NS-1, NS-2, NS-3 and NS-5 of the hepatitis C virus (HCV), each arranged in a specific sequence, has been identified and made by solid phase peptide synthesis. These peptides have been found to be useful for the detection of antibodies to HCV in sera and body fluids and for the diagnosis of non-A, non-B hepatitis (NANBH). Because of their immunoreactivity, it is expected that these peptides are also useful in stimulating production of antibodies to HCV in healthy mammals such as Balb/C mice, and in a vaccine composition to prevent HCV or NANBHV infection.

According to the present invention, a peptide composition useful for the detection of antibodies to HCV and diagnosis of NANBH comprises a peptide from the envelope, NS-1, NS-2, NS-3 and NS-5 regions of the HCV represented by the following sequences:

(a) Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-Val-Tyr-Cys-X

Pep1

(b) Pro-Pro-Leu-Gly-Asn-Trp-Phe-Gly-Cys-Thr-Trp-Met-Asn-Ser-Thr-Gly-Phe-Thr-Lys-Val-Cys-Gly-Ala-Pro-Pro-Cys-X

Pep2

(c) Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Leu-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-X

Pep3

(d) Asp-Pro-Ser-His-Ile-Thr-Ala-Glu-Ala-Ala-Gly-Arg-Arg-Leu-Ala-Arg-Gly-Ser-Pro-Pro-Ser-Val-Ala-Ser-Ser-Ser-Ala-Ser-Gln-Leu-Ser-Ala-Pro-Ser-Leu-Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-X

Pep4

(e) Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X

Pep5

(f) Asp-Pro-Gln-Ala-Arg-Val-Ala-Ile-Lys-Ser-Leu-Thr-Glu-Arg-Leu-Thr-Val-Gly-Gly-Pro-Leu-Thr-Asn-Ser-Arg-Gly-Glu-Asn-Cys-Gly-Tyr-Arg-Arg-Cys-Arg-Ala-Ser-X

Pep6

(g) Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-Ssr-Arg-Cys-X

Pep7

(h) Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala-X

Pep8

(i) Val-Asp-Ala-Glu-Thr-Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn-X

Pep9

(j) Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Lsu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Glu-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys-X

Pep10

(k) Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr-X

Pep11

(l) Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X

Pep12

(m) Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-X

Pep13

(n) Cys-Lys-Pro-Leu-Leu-Arg-Glu-Glu-Val-Ser-Phe-Arg-Val-Gly-Leu-Hie-Glu-Tyr-Pro-Val-Gly-Ser-Gln-Leu-Pro-Cye-Glu-Pro-Glu-Pro-Aep-X

Pep14

(o) Glu-Glu-Tyr-Val-Glu-Ile-Arg-Gln-Val-Gly-Asp-Phe-His-Tyr-Val-Thr-Gly-Met-Thr-Thr-Asp-Asn-Leu-Lys-Cys-Pro-Cys-Gln-Val-Pro-Ser-Pro-X

Pep15

(p) Gly-Ser-Trp-Leu-Arg-Asp-Ile-Trp-Asp-Trp-Ile-Cys-Glu-Val-Leu-Ser-Asp-Phe-Lys-Thr-Trp-Leu-Lys-Ala-Lys-Leu-Met-Pro-Gln-Leu-X

Pep16

(q) Gly-Pro-Ala-Asp-Gly-Met-Val-Ser-Lys-Gly-Trp-Arg-Leu-Leu-Ala-Pro-Ile-Thr-Ala-Tyr-Ala-Gln-Gln-Thr-Arg-Gly-Leu-Leu-Gly-Cys-Ile-Ile-Thr-Ser-Leu-Thr-Gly-Arg-Asp-Lys-Asn-Gln-Val-Glu-Gly-X

Pep17

(r) Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-Cye-His-Ser-Lys-Lys-Lys-Cye-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Val-Ala-Leu-X

Pep18

(s) Cye-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cye-Trp-Val-Ala-Met-Thr-Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys-X

Pep19

These 19 peptides are in addition to Peptide VIIIE, a peptide from the structural protein region, and Peptides IIH and V, peptides from the non-structural protein region which have also been found to be reactive and useful for the detection of antibodies to HCV and diagnosis of NANBH.

Peptide VIIIE has the following sequence:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thy-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X,

Peptide IIH has the following sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

Peptide v has the following sequence:

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

wherein X is -OH or -NH_2 and analogues, segments, mixtures, conjugates and polymers thereof.

Further, according to the present invention, the peptides by themselves, or when coupled to a protein or a polymeric carrier of homo or hetero dimers or higher oligomers by the use of homo or hetero functional multivalent cross linking reagents, or when directly synthesized and conjugated to a branching polyvalent lysine resin, can be used to elicit the production of antibodies to HCV in healthy mammals, including humans.

The method comprises introducing an effective amount of the peptide composition containing each of the individual peptides, analogues or segments or a mixture or a combination thereof, or in a polymeric form, into the body of a healthy mammal by intraperitoneal or subcutaneous injection.

Vaccines containing the peptides according to the present invention as the key immunogen may also be prepared as described above or by known methods. It is expected that such vaccine compositions may be useful to prevent HCV infection or NANBH.

BRIEF DESCRIPTION OF DRAWING

Fig. 1 is a photograph of a computer-generated structure of an octameric peptide immunogen.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, nineteen peptides and their analogues including segments have been selected from the nonstructural regions of HCV and chemically synthesized. These peptides including their analogues are useful for the detection of antibodies to HCV in body fluids, the diagnosis of NANBH, and for the vaccination of healthy mammals by stimulating the production of antibodies to HCV. These peptides are arranged in the following sequences:

(a) Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-Val-Tyr-Cys-X

Pep1

(b) Pro-Pro-Leu-Gly-Asn-Trp-Phe-Gly-Cys-Thr-Trp-Met-Asn-Ser-Thr-Gly-Phe-Thr-Lys-Val-Cys-Gly-Ala-Pro-Pro-Cys-X

Pep2

(c) Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Leu-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-X

Pep3

(d) Asp-Pro-Ser-His-Ile-Thr-Ala-Glu-Ala-Ala-Gly-Arg-Arg-Leu-Ala-Arg-Gly-Ser-Pro-Pro-Ser-Val-Ala-Ser-Ser-Ser-Ala-Ser-Gln-Leu-Ser-Ala-Pro-Ser-Leu-Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-X

Pep4

(e) Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X

Pep5

(f) Asp-Pro-Gln-Ala-Arg-Val-Ala-Ile-Lys-Ser-Leu-Thr-Glu-Arg-Leu-Thr-Val-Gly-Gly-Pro-Leu-Thr-Asn-Ser-Arg-Gly-Glu-Asn-Cys-Gly-Tyr-Arg-Arg-Cys-Arg-Ala-Ser-X

Pep6

(g) Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-X

Pep7

(h) Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala-X

Pep8

(i) Val-Asp-Ala-Glu-Thr-Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn-X

Pep9

(j) Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Glu-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys-X

Pep10

(k) Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr-X

Pep11

(l) Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X

Pep12

(m) Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-

Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-X

Pep13

(n) Cys-Lys-Pro-Leu-Leu-Arg-Glu-Glu-Val-Ser-Phe-Arg-Val-Gly-Leu-His-Glu-Tyr-Pro-Val-Gly-Ser-Gln-Leu-Pro-Cys-Glu-Pro-Glu-Pro-Asp-X

Pep14

(o) Glu-Glu-Tyr-Val-Glu-Ile-Arg-Gln-Val-Gly-Asp-Phe-His-Tyr-Val-Thr-Gly-Met-Thr-Thr-Asp-Asn-Leu-Lys-Cys-Pro-Cys-Gln-Val-Pro-Ser-Pro-X

Pep15

(p) Gly-Ser-Trp-Leu-Arg-Asp-Ile-Trp-Asp-Trp-Ile-Cys-Glu-Val-Leu-Ser-Asp-Phe-Lys-Thr-Trp-Leu-Lys-Ala-Lys-Leu-Met-Pro-Gln-Leu-X

Pep16

(q) Gly-Pro-Ala-Asp-Gly-Met-Val-Ser-Lys-Gly-Trp-Arg-Leu-Leu-Ala-Pro-Ile-Thr-Ala-Tyr-Ala-Gln-Gln-Thr-Arg-Gly-Leu-Leu-Gly-Cys-Ile-Ile-Thr-Ser-Leu-Thr-Gly-Arg-Asp-Lys-Asn-Gln-Val-Glu-Gly-X

Pep17

(r) Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp--Glu-Leu-Ala-Ala-Lys-Leu-Val-Ala-Leu-X

Pep18

(s) Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys-X

Pep19

These 19 peptides are in addition to Peptide VIIIE, a peptide from the structural protein region, and Peptides IIH and V, peptides from the non-structural protein region which have also been found to be reactive and useful for the detection of antibodies to HCV and diagnosis of NANBH.

Peptide VIIIE has the following sequence:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X,

Peptide IIH has the following sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

Peptide V has the following sequence:

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

Wherein X is -OH or -NH_2 and analogues, segments, mixtures, conjugates, and polymers thereof.

These peptides may comprise combinations or segments, i.e. longer or shorter peptide chains by having more amino acids added to the terminal amino acids, or by amino acids removed from either terminal end.

These peptides may also comprise analogues to accommodate strain-to-strain variations among different isolates of HCV. HCV is indicated to have frequent mutations. Therefore, it is expected that variant strains, such as PT, J, J-1 and J-4 (1-4) exist. Adjustments for conservative substitutions and selection among the alternatives where non-conservative substitutions are involved, may be made in the prescribed sequences (e.g. see Table 1E, Table 8c and Table 11 for possible amino acid substitutions in the hypervariable regions of the envelope and NS-1 proteins). These analogues of the synthetic peptides may therefore comprise substitutions, insertions and/or deletions of the recited amino acids of the above sequence to accomodate the various strains, as long as the immunoreativity recognizable by the antibodies to HCV is preserved

These peptides may also comprise conjugates, i.e., they may be coupled to carrier proteins such as bovine serum albumin (BSA) or human serum albumin (HSA). Furthermore, these peptides may comprise polymers, i.e., they may be synthesized on a polymeric resin or in dimeric, tetrameric, octameric and decahexyl forms of the peptide or their analogues, such as a branching octameric lysine resin.

The branchine poly-L-lysine can be Lys_8 Lys_4 Lys_2 Lys, Lys_4 Lys_2 Lys, Lys_2 Lys,Lys; the last Lys can be attached to Y as in Lys_4 Lys_2 Lys-Y wherein Y is -OH, -NH_2 or an amino acid containing no side chain functional group, such as alanine, valine, glycine, etc. Y can be inserted to facilitate synthesis onto the 4-methylbenzylhydrylamine resin. The conjugates and polymers of the peptides are also useful in the present invention.

The amino acid sequences of the polypeptide as described in the invention useful as test reagents for the detection of antibodies to HCV in body fluids and diagnosis of NANBH are selected to correspond to segments of the amino acid sequence of the postulated envelope and non-structural proteins of HCV designated as env, NS-1, NS-2, NS-3 and NS-5 based on amino acid sequence information derived from Houghton et al. (13), Okamoto et al (2) and Kato et al (4).

In selecting regions of the HCV protein for epitope analysis, peptides of about 40 mer size with amino acid sequences covering the complete HCV envelope and non-structural proteins NS-1, NS-2, NS-3 and

NS-5 were synthesized. These were tested for their immunoreactivity with special specimens previously selected through the screening of thousands of patient and normal sera for their unique immunoreactivity with HCV. Nineteen peptides from the postulated envelope and nonstructural protein regions NS-1, NS-2, NS-3 and NS-5 designated as pep1, pep2, pep3, pep4, pep5, pep6, pep7, pep8, pep9, pep10, pep11, pep12, pep13, pep14, pep15, pep16, pep17, pep18 and pep19 and their analogues were identified to have specific immunoreactivity with the positive HCV sera.

At present, available knowledge of protein structure has not enabled the scientist to predict the amino acid sequences that may represent highly immunogenic epitopes. The usefulness of a peptide as an antigen or immunogen must be empirically determined. We have only been able to identify and characterize immuno-reactive epitopes through an extensive process which we call "serological validation". The following example shows how difficult it is to identify immuno-reactive epitopes.

For example, a clone designated as C33c encoded within the NS-3 region was reported to possess immunoreactivity(3). This clone spans 265 amino acid residues. Assuming a useful peptide must be at least 6 amino acids in length and that the upper limit for synthetic peptides in reasonable yield is 120 residues, the number of possible unique peptides from the C33c regions is 23,028. For the entire HCV genome, the figure is about 260,000.

In addition, we have shown that extraction conditions are critical for the expression of the immunopotency of a peptide (Example 4C), so the number of uniquely extracted peptides from this region is in multiples of 23,028. The possibilities for post-extraction modification, such as pH adjustment (Example 4B) further increase the possible selections to $>10^6$. If amino acid substitutions at various positions are taken into consideration, this figure will quickly increase to several millions. In contrast to the HCV core region, in which peptides VIIIE and IXD were the optimal analogues, longer peptides are not preferred over shorter analogues in the NS-3/C33c region. For example, the 42 mer 279B shown on Table 4D has only 3% of the reactivity of the 37 mer peptide 3, designated es 279A in Table 4D. Of 30 peptides spanning the C33c region tested, only one was found to be useful. The antigenic index as referred in Houghton et al (3) did not prove to be a useful guide to epitopes, as the profile for peptide 3 is positive for only 30% of its sequence and negative for the remaining 70%.

The stretegy for serological validation also depends on the expected characteristics of the target epitopes. Universal immunodominant epitopes, such as the gp41 transmembrane peptide of HIV-1, may be screened by a single representative serum sample from a patient known to be infected with the virus. Epitopes which are not recognized by all infected individuals, or those for which antibody is produced late or only transiently, and especially epitopes which give rise to neutralizing antibodies, must be screened by large panels of sera. For example, peptide 272B shown in Table 4A was initially tested on a panel of eight sera from HCV infected individuals (Panel 1). Only one sample was definitely positive with an absorbance of 880 mA. Three were weakly reactive (<200 mA) and four were negative.

The identification of the immuno-reactive epitopes is also dependent on the panel of sera used. The more closely the penel represents the population most likely to be seropositive for the desired epitope, the greater the chance that the epitope will be identified. For example, peptides synthesized from the NS-1 region, which were hypothesized to be important for generating neutralizing antibodies, gave only weakly reactive or negative results on screening with a very large number (n > 200) of samples from individuals who were newly Infected and/or chronically infected with HCV. However, a panel of 24 samples from asymptomatic individuals from a known hepatitis virus endemic geographical region, Taiwan and mainland China, yielded two samples with absorbances of >2000 mA against multiple NS-1 peptides.

Finally, if the desired purpose of a targeted peptide/epitope is to extend the range of reactivity of an assay comprised of previously identified epitopes, then a large number of samples from individuals at risk of infection but seronegative against known epitopes must be employed for screening. Unfortunately, the most critical samples from clinically proven and documented cases of infection mey be available in quantities insufficient for screening purposes. This is another complication/difficulty encountered in serological validation for determining the immunoreactivity of a peptide.

The process of "serological validation" is particularly difficult when the epitopes to be identified elicit antibodies only in a subpopulation of an infected patient group. When such epitopes become targets for identification, special attention must be paid to synthetic peptides which show very weak reactivity when tested by an enzyme immunoassay.

Fortunately, the low background absorbance of synthetic peptides allows for the precise detection of weak reactivities. In some cases, absorbences of 50 mA versus background reeding are of sufficient significance and can lead to the identification of important epitopes through successive refinement of the amino acid sequence of a peptide. The utmost technical skill is required to obtain consistent and reliable results when working in the range of absorbances below 200-300 mA. For example: Peptides 261E end

261F shown on Table 4D were reactive with only one of eight HCV sera panel members (Panel I), with absorbances of 307 and 269 mA, respectively. Yet this weak reactivity led to the eventual identification of pep3 (or 279A), toward which half of the panel is reactive, and toward which some additional reactive samples show absorbances of >2000 mA.

Based on the immunoreactivities of the peptides according to the present invention, it is believed that these peptides may also be useful in a vaccine to prevent NANBH. The peptide when coupled to a protein, or synthesized on a polymeric carrier resin (e.g., an octameric lysine resin) or when polymerized to homo or hetero dimers or higher oligomers by cysteine oxidation, or induced disulfide cross linking, or by use of homo or hetero functional multivalent cross linking reagents, can be introduced to normal subjects to stimulate production of antibodies to HCV in healthy mammals.

The advantages of using synthetic peptides are known.

Since the peptides according to the present invention are not derived biologically from the virus, there is no danger of exposing the normal subjects who are to be vaccinated to the disease causing pathogen.

The peptides can be chemically synthesized easily. This means that there is no involvement with HCV at any time during the process of making the test reagent or the vaccine. Another problem which can be minimized by the process of the present invention is the false positive results caused by the presence of antigenic material co-purified with the HCV fusion protein. Certain normal individuals have antibodies to E. coli or yeast proteins which are cross reactive with the antigenic materials from the expression system. Sera from these normal individuals may show a positive reaction in the immunoassays.

Further, with appropriate amino acid modification or substitutions, it is expected that various peptide analogues based on the prescribed amino acid sequence can be synthesized with properties giving rise to lower background readings or better binding capacity to solid phases useful for HCV antibody screening assays.

Moreover, because the peptide compositions of the present invention are synthetically prepared, the quality can be controlled and as a result, reproducibility of the test results can be assured. Also, since very small amounts of a peptide are required for each test procedure, and because the expense of preparing a peptide is relatively low, the cost of screening body fluids for antibodies to HCV, diagnosis of NANBH infection, and the preparation of a vaccine is relatively low.

The peptides prepared in accordance with the present invention can be used to detect HCV infection and diagnose NANBH by using them as the test reagent in an enzyme-linked immunoadsorbent assay (ELISA), an enzyme immunodot assay, an agglutination based assay, or other well-known immunosassay devices. The following examples serve to illustrate the present invention and are not to be used to limit the scope of the invention.

EXAMPLE 1

Measurement of Relative (%) Immunoreactivity for HCV synthetic peptides by an Enzyme-Linked Immunosorbent Assay

As an example to illustrate how relative (%) immunoreactivity for HCV synthetic peptides is measured, wells of 96-well plates are coated for 1 hour at 37°C, with each of the following peptides: IIH, V, VIIIE and pep11 at 5 ug/mL at 100 uL per well in 10mM $NaHCO_3$ buffer, pH 9.5. The peptide coated wells were then incubated with 250 uL of 3% by weight of gelatin in PBS in 37°C for 1 hour to block non-specific protein binding sites, followed by three washes with PBS containing 0.05% by volume of TWEEN 20 and then dried. The test specimens containing a panel of eight well-characterized HCV antibody positive patient sera were diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20 volume to volume, respectively. 200 uL of the diluted specimens were added to each of the wells and allowed to react for 15 minutes at 37°C.

The wells were then washed six times with 0.05% by volume TWEEN 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase conjugated goat anti-human IgG was used as a second antibody tracer to bind with the HCV antibody-peptide antigen complex formed in positive wells. 100 uL of peroxidase labeled goat anti-human IgG at a dilution of 1:1B00 in 1% by volume normal goat serum, 0.05% by volume TWEEN 20 in PBS was added to each well and incubated at 37°C for another 15 minutes.

The wells were washed six times with 0.05% by volume TWEEN 20 PBS to remove unbound antibody and reacted with 100uL of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.12% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0.

This substrate mixture was used to detect the peroxidase label by forming a colored product. Reactions were stopped by the addition of 100 uL of 1.0M H_2SO_4 and the A_{492}mm measured. Results of relative

immunoreactivity for each of the peptides obtained from this study are shown in Table A using peptide II H as the reference.

Table A

Peptide Code	A_{492}nm (Panel I, No. 1 to 8)									
	1	2	3	4	5	6	7	8	Total	%
IIH	0.812	0.656	3.114	2.737	1.066	2.254	2.599	3.478	16.712	100
V	0.834	1.060	2.931	0.534	0.137	0.434	0.303	2.787	9.020	54
VIIIE	2.745	2.208	2.468	3.032	0.054	2.108	0.730	3.006	16.351	98
Pep11	0.241	0.715	3.162	1.020	0.568	2.166	3.330	3.477	14.690	88

EXAMPLE 2

Comparison of HCV Immunoreactivities by a Well-characterized 8 Member HCV Serum Panel (Panel I) for % Relative Immunoreactivity with a Group of HCV Capsid Protein Related Peptides by an Enzyme Immunoassay

A 36mer HCV capsid peptide recently disclosed by Okamoto et al. (8) as the basis of an HCV EIA was synthesized for the purpose of comparison of immunoreactivity with peptides VIIIA, VIIIB and VIIIE (Table 2A). According to a procedure described in Example 1, peptides were coated at concentrations of 5, 1 and 0.2 µg/mL for immunopotency comparison. This 36mer exhibited only 47.8% of the reactivity of VIIIE (Table 2A). More importantly, when tested by our well-characterized HCV serum panel used lor serological validation, only 4 out of 8 samples reacted with the 36mer, compared with 7 out of 8 with VIIIE. The C terminal end of this 36mer does not appear to contribute to the peptide's HCV immunoreactivity, since IXD is not greater in reactivity than IXC (Table 2A).

In addition, a 61mer peptide and fragments thereof consisting of a 30mer, a 40mer and a 50mer corresponding to sequences from Arima clone 1, which is homologous to the capsid reglon of the flavivirus yellow fever virus, were synthesized and compared in immunoreactivity with peptide VIIIE from the corresponding region of HCV (Table 2B). The 40mer and 61mer ol clone 1 exhibited the most reactivity. However these were only 21.1% and 20.7%, respectively, of the immunoreactivity of peptide VIIIE.

Table 2A

		% Relative Immunoreactivity
Okamoto et al.(8)(36mer)	RRGPRLGVRATRKTSERSQPRGRRQPIPKVRRPEGR	47.8%
VIII A	GPRLGVRATRKTSERSQPRGRR	32.7%
VIII B	VGGVYLLPRRGPRLGVRATRKTSERSQPRGPR	48.9%
VIII E	STIPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRR	100.0%
IX C	TWAQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTDPRRRSRNLG	57.9%
IX D	IPKVRRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTDPRRRSRNLG	58.9%
IX E	GRRQPIPKVRRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTDPRRRSRNLG	50.2%

Table 2B

		% Relative Immunoreactivity
Arima et al. (12)		
30 mer	PGKNKKPRVGRIQKMMREGRKDAYQIRKRR	0.7%
40mer	KEKEKTATNWPGKNKKPRVGRIQKMMREGRKDAYQIRKRR	21.1%
50mer	NDTNKKQRRTYKEKEKTATNWPGKNKKPRVGRIQKMMREGRKDAYQIRKRR	17.8%
61mer	KKGEASMGEAENDTHKSQRRYKEKEKTATNWPGKNKKPRVGRIQKMMREGRKDAYQIRKRR	20.7%

EXAMPLE 3

Relative (%) Immunoreactivity for NS-1 Synthetic Peptides by an Enzyme-Linked Immunosorbent Assay

(A) Identification of Immunoreactive NS-1 Peptides.

Wells of 96-well plates were coated for 1 hour at 37° C with each of the 16 peptides (designated as peptides 241A-C, 231A-E, 232A-D, 233C, 234A-C), synthesized according to sequences derived from the NS-1 region (Table 3A), at 5 ug/mL at 100 uL per well in 10 mM $NaHCO_3$ buffer, pH 9.5. Each peptide's immunoreactivity was measured as previously described (see Example 1), using an 8 member serum panel (Panel I).

All sixteen peptides showed little or no reactivity with serum panel I. The most reactive peptide, pep1 (designated 231c in Table 3A), had an immunopotency index of 13.9%, compared with peptide VIIIE on the same panel. There were isolated examples of epitope recognition; for example, for sample 4, all analogues of the 232 series had absorbances less than or equal to 20 mA except for the longest peptide, 232D, which had an absorbance of 785 mA. However, the remaining 7 panel members were negative when tested with 232D.

After screening these 16 NS-1 region derived peptides with more than 200 additional HCV positive sera with little or no demonstrated immunoreactivities, immunoreactivities of these 16 NS-1 peptides with other sera were sought. A panel of serum samples from individuals coming from regions in which hepatitis C is endemic, namely mainland China and Taiwan, were tested for evidence of reactivity to these NS-1 protein derived peptides. Twenty-four samples were chosen from individuals who had no recognizable symptoms of non-A, non-B hepatitis and for whom the peptide based HCV EIA, Formet C, as described in Example 11, was nonreactive. Seven of the 24 samples (29%) were reactive against one or more peptides from the NS-1 region, indicative of the presence of long term protective antibodies responsive to this region. This 7 member panel (designated as Panel II, CH1-CH7) was used to further characterize these NS-1 peptides for their immunoreactivity.

The peptide with the greatest reectivity against the serum Panel II again was pep1 (designated 231c in Table 3A). Using this peptide as e standard, the relative immunoreactivity for each of the other 15 peptides from the NS-1 region are calculated in Table 3A.

Detailed results from the seven member serum Panel II on four of the most immunoreactive analogues (i.e. pep1, or 231C; pep2, or 232A; 233C end 234A) are tabulated in Table 3B. The reactivities of 231C and 232A are complementary in that CH-1 and CH-2 are strongest on 231C, whereas CH-3 through CH-7 are stronger on 232A.

(B) NS-1 Reactivity in Early and Long-term HCV Infection.

In addition, all sixteen NS-1 peptides were tested on panels of samples representing HCV-antibody positive donors (n = 9) in an early stage of infection, namely plasmapheresis donors with the first occurrence of an ALT level >100 i.u./L, and those asymptomatic individuals (n = 14) disqualified from blood donation because of a reactive result for anti-HIV or HBc, for whom the anti-HCV result probably represents a past infection. These select panels were chosen from hundreds of HCV positive sera for their ability to recognize NS-1 antigens. The results of testing the panels with the 16 NS-1 peptides are given in Table 3C. For both groups, peptide designated as 232A (pep2) had the greatest immunoreactivity. Using pep2 as a standard, the relative immunoreactivity of each peptide was calculated (Table 3C).

Table 3A

Synthetic Peptides with their Amino Acid Sequences derived from the HCV NS-1 Protein Region

	CPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVCGPVYCFTPSPVVVGTTDRSGAPTYSWGENDTDVFVLNNTRPPLGNWFGCTWMNSTGFTKVCGAPPC	% IP
241A	QGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVC	63.9
241B	CRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVC	92.9
241C	CPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVC	93.6
231A	RPYCWHYPPKPCGIVPAKSVCGPVYC	83.2
231B	ANGSGPDQRPYCWHYPPKPCGIVPAKSVCGPVYC	96.4
231C(Pep1)	QGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVCGPVYC	100.0
231D	CRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVCGPVYC	65.3
231E	CPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAKSVCGPVYC	87.6
232A(Pep2)	PPLGNWFGCTWMNSTGFTKVCGAPPC	88.7
232B	VFVLNNTRPPLGNWFGCTWMNSTGFTKVCGAPPC	82.9
232C	SWGENDTDVFVLNNTRPPLGNWFGCTWMNSTGFTKVCGAPPC	27.9
232D	DRSGAPTYSWGENDTDVFVLNNTRPPLGNWFGCTWMNSTGFTKVCGAPPC	25.9
	VIGGAGNNTLHCPTDCFRKHPDATYSRCGSGPWITPRCLVDYPYRLWHYPCTINYTIFKIRMYVGGVEHRLEAACNWTRGERCDLEDRDRSELS	
233C	LHCPTDCFRKHPDATYSRCGSGPWITPRCLVDYPYRLWHYPC	16.8
234A	EAACNWTRGERCDLEDRDRSELS	20.5
234B	VGGVEHRLEAACNWTRGERCDLEDRDRSELS	17.9
234C	TIFKIRMYVGGVEHRLEAACNWTRGERCDLEDRDRSELS	8.8

Table 3B

Sample No. (Panel II)	A492nm by EIA (mA)			
	231C (Pep1)	232A (Pep2)	233C	234A
CH-1	2237	202	123	118
CH-2	2472	261	174	232
CH-3	171	935	72	64
CH-4	218	1498	238	227
CH-5	311	621	114	206
CH-6	247	1128	175	202
CH-7	206	552	89	151

Table 3C

Panel I.D. Panel Size	Early HCV Infection n = 9	Late HCV Infection n = 14
Peptide Code	% Relative Immunoreactivity in comparison to Pep2 (232A)	
241A	23.9	43.8
241B	32.7	75.D
241C	44.7	84.7
231A	46.8	48.4
231B	30.9	46.7
231C (Pep1)	88.6	62.7
231D	23.3	43.5
231E	70.9	83.4
232B	91.4	83.5
232C	21.7	22.D
232D	50.0	46.7
233A	9.9	17.5
234A	9.5	15.7
234B	13.2	20.9
234C	12.1	44.5

EXAMPLE 4

Relative (%) Immunoreactivity for NS-3 Protein Derived Synthetic Peptides by an Enzyme-Linked Immunosorbent Assay

(A) Identification of NS-3 Protein Derived Immunoreective Peptides.

Wells of 96-well plates were coated for 1 hour at 37° C with each of the 30 peptides (designated as 261A-F, 262A-F, 272A-C, 274A-D, 275A-D, 278A-D and 279A,B,E), synthesized with sequences derived from the NS-3 region, at 5 ug/mL at 100 uL per well in 10 mM $NaHCO_3$ buffer, pH 9.5. The immunoreactivity of each peptide was measured by an 8 member HCV serum panel (Panel I). The peptide with the greatest immunoreactivity, pep3, designated 279A In Table 4D, had a relative immunoreactivity value of 23.9%, compered with peptide VIIIE (data not shown). When the immunoreactivity of peptide 3 was used es a stendard to calculate the relative immunopotency for the other NS-3 peptides (Tebles 4A, 4B, 4C and 4D), all other 29 peptides were found to be marginally immunoreactive. More surprisingly, the sequence of pep3 (or 279A), a 37mer, is entirely contained within peptides 261E, 261F, 274B, 274C, 274D, 279B and 279E, yet these seven larger peptides have relative immunoreactivity in the range of only 2.2 to 34%, when compared to thelr segment pep3. Another surprise was the observation that the mere addition of 5 resldues to the N terminus of pep3 completely abrogates the reactivity of the peptide (see the relative immunoreactivity of pep3 vs. peptide 279B, Table 4D).

Table 4A

HCV NS-3 PROTEIN DERIVED SYNTHETIC PEPTIDES

	AVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGV	% IP
272A	PVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPS	2.1
272B	TTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPS	38.0
272C	AVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPS	11.8
278A	PVVPQSFQVAHLHAPTGSGKSTKVPAAYA	8.2
278B	FTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYA	1.4
278C	VENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSYKVPAAYA	10.7
278D	AVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYA	11.6

Table 4B

HCV NS-3 PROTEIN DERIVED SYNTHETIC PEPTIDES

	YMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITYGSPITYSTYGKFLADGGCSGGAYDIIICDECHS	% Relative Immunoreactivity
275A	RTITTGSPITYSYYGKFLADGGCSGGAYDIIICDECHS	18.2
275B	KVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRYITTGSPITYSTYGKFLADGGCSGGAYDIIICDECHS	11.1
275C	HLNAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTIYTGSPITYSTYGKFLADGGCSGGAYDIIICDECHS	9.6
275D	YMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFLADGGCSGGAYDIIICDECHS	5.4

Table 4C

HCV NS-3 PROTEIN DERIVED SYNTHETIC PEPTIDES

		% Relative Immunoreactivity
274A	GYKVLVLNPSVAATLGFGATMSKAHGIDPNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVAL	2.0
274B	TVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVAL	34.0
274C	GCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVAL	3.9
274D	AHGIDPNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVAL	2.8
	GYKVLVLNPSVAATLGFGATMSKAHGIDPNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVAL	

Table 4D

HCV NS-3 PROTEIN DERIVED SYNTHETIC PEPTIDES

		% Relative Immunoreactivity
262A	TGKFLADGGCSGGAYDIIICDECHSTDAT	6.9
262B	TTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDAT	4.7
262C	PNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDAT	9.3
262D	AYMSKAHGIDPNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDAT	3.6
262E	SVAATLGFGAYMSKAHGIDPNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDAT	4.7
262F	GTKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTIITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDAT	5.1

		% Relative Immunoreactivity
261A	RTITTGSPITYSTTGKFLADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFTGKAIPLEVIKGGRHLIFCHSKKKCDEL	7.2
261B	PFTGKAIPLEVIKGGRHLIFCHSKKKCDEL	3.1
261C	EVALSTTGEIPFTGKAIPLEVIKGGRHLIFCHSKKKCDEL	3.2
261D	SVTVPHPNIEEVALSTTGEIPFTGKAIPLEVIKGGRHLIFCHSKKKCDEL	15.8
261E	TVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFTGKAIPLEVIKGGRHLIFCHSKKKCDEL	14.9
261F	GGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFTGKAIPLEVIKGGRHLIFCHSKKKCDEL	21.4
279A (Pep3)	GCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAG	100.0
279B	FLADGGCSGGATDIIICDECHSTDATSILGIGTVLDQAETAG	3.0
279E	RTITTGSPITYSTTGKFLADGGCSGGATDIIICDECHSTDATSILGIGTVLDQAETAG	2.2

(B) Enhancement of Peptide Immunoreactivity by pH Adjustment.

Although the Immunoreactivities of 29 of the 30 NS-3 derived peptides, as originally synthesized and cleaved products, were marginal, the conformation of some peptides could be modulated by pH adjustment

to enhance their immunoreactivity.

Peptides dissolved at 1 mg/mL in H_2O, pH 4, were titrated to pH 11 by addition of diluted NaOH. After 5 min at pH 11, the pH of the peptide solution was brought down to 7.0 using diluted HCl. Immunoreactivity of the peptides thus treated was compared with reactivity prior to pH adjustment (Table 4E). Two- to three-fold increases in A492nm were seen. Some previously non-reactive serum samples were able to react with pH adjusted peptides. For instance, serum sample 1, which is non-reactive to 261C, has an absorbance of 1401 mA when tested with the corresponding pH adjusted peptide. Adjustment of pH increases the relative immunopotency of peptide 261C from 3.2% to 68.5%, compared with the standard pep3 (or 279A).

(C) Effect of Extraction Conditions after HF Cleavage on the Immunoreactivities of Peptides.

Peptide extraction conditions after HF cleavage were altered to test for their effect on peptide immunopotency after HF cleavage. Pep3 (or 279A) was extracted with acetic acid at pH 2, whereas pep3' was extracted with ammonium bicarbonate at pH 8. The latter extracted product showed a decrease in its reactivity in all reactive samples tested (Table 4F). The decrease ranged from 77.6% to 99.3%.

Table 4E

	A492nm (mA) by EIA							
	274B		275B		261C		272C	
	Ctrl	pH adj	Ctrl	pH adj	Ctrl	pH adj	Ctrl	pH adj
1	628	1604	210	591	6	1401	8	973
2	148	466	37	159	5	499	74	255
3	9	625	0	217	24	175	29	141
4	464	1144	124	311	27	351	17	158

Table 4F

Effect of Extraction Conditions on Synthetic Peptide's Immunopotency

	A492nm (mA) by EIA		% Decrease
	Pep3 Acetic Acid	Pep3' $(NH_4)_2CO_3$	
Blank	0	0	-
NRC	1	1	-
WRC	565	59	89.6
SRC	2213	495	77.6
#1	1550	329	78.8
#2	628	63	90.0
#3	1323	112	91.5
#4	1019	7	99.3
#5	1610	193	88.0

NRC: Negative Control
WRC: Weakly Reactive Control
SRC: Strongly Reactive Control

EXAMPLE 5

Relative (%) Immunoreactivity for NS-5 Protein Derived Synthetic Peptides by an Enzyme-Linked Immunosorbent Assay

Wells of 96-well plates were coated for 1 hour at 37° C with each of the three peptides derived from the NS-5 region of HCV (designated as pep4, pep5 and pep6). The results obtained (Table 5) show that all these peptides were immunoreactive with a unique group of 5 HCV positive sera.

Table 5

HCV NS-5 Protein Derived Synthetic Peptides

Code	Amino Acid Sequence	% Relative Immunoreactivity
Pep4	DPSHITAEAAGRRLARGSPPSVASSSASQLSAPSLKATCTANHDSP	28.6
Pep5	DAELIEANLLWRQEMGGNITRVESENKVVTLDSFDPLVAEEDER	100.0
Pep6	DPQARVAIKSLTERLTVGGPLTNSRGENCGYRRCRASRAS	17.0

Sample No.	Pep4	Pep5	Pep6
1	0.468	2.942	0.550
2	0.659	0.370	0.245
3	0.675	0.616	0.043
4	0.063	1.316	0.162
5	0.144	1.783	0.192

EXAMPLE 6

Detection of Antibodies to HCV By an Agglutination Based Assay

The presently claimed HCV peptides, synthesized according to the Merrifield solid phase method, can be conjugated to bovine serum albumin (BSA) by a simple crosslinking method in the presence of a low percentage of glutaraldehyde solution, or with other crosslinking reagent such as m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS).

Based on the above mentioned peptide-BSA conjugation process, conjugated peptide was absorbed onto double aldehyde fixed human O erythrocytes at pH 4.0. The peptide-conjugate coated erythrocytes were then treated with $NaBH_4$ to prevent non-specific protein binding. The peptide-conjugate coated erythrocytes were then washed with PBS end incubated with 5% normal human serum-PBS solution. These

processed cells were then used in an agglutination assay for the detection of HCV antibodies in both serum and plasma specimens. The specimens were diluted 1:10 in a sample diluent buffer and an equal volume of the indicator cells was mixed with the diluted specimens. The agglutination pattern was settled within one hour; and the assay results were read by eye. Serial bleedings from three well-characterized HCV seroconversion panels were tested for antibodies to HCV in the above-described HCV passive hemagglutination assay (PHA) employing Peptide VIIIE-BSA conjugate and Peptide IIH-BSA conjugate as the solid phase. The results were compared with the A492 and S/C of the peptide based HCV EIA (Format C, as described in Example 11) and C100 based HCV EIA (Teble 6).

In brief, the PHA assay detected HCV entibodies in all three panels as early as there was an increase in A492 in the peptide based EIA (Format C). rC100 based EIA lagged behind the HCV PHA results by 4-8 weeks.

Table 6

Oectection of HCV Specific Antibodies from Seroconversion Panels by Various HCV Antibody Assays

Series	Days	ALT	Format C HCV EIA S/C Ratio	C100 Based HCV EIA	HVC PHA Visual Score
A* (Serologicals Panel B)	0	40	0.108	0.03	-
	7	32	0.045	0.04	-
	14	32	0.025	0.06	++
	21	180	1.037	0.04	++
	50	401	7.193	0.19	++
	92	-	10.185	6.57	++
	105	-	9.770	6.57	++
B* (Serologicals Panel A)	0	39	0	0	-
	10	274	0.058	0	-
	14	346	0.128	0	-
	30	1175	7.835	6.5	++
	51	430	7.811	6.5	++
C* (Serologicals Panel C)	0	63	0.115	0.04	-
	2	81	1.607	0.04	++
	9	183	2.506	0.02	++++
	29	563	9.827	6.57	++++
	57	436	10.630	6.57	++++

* Case presented is a plasma donor from a commercial source. Day 0 designates first sample in the series and does not correspond to date of exposure.

Example 7

Detection of Antibodies to HCV by an Agglutination Assay Utilizing as the Solid Phase Immunosorbent Latex Particles Coated with HCV Peptide

Using the peptide-BSA conjugation process mentioned in the previous example, conjugated peptide VIIIE-BSA, was absorbed to latex particles (0.4μ size) at pH 9.5. The peptide-conjugate coated latex particles were then treated with BSA to prevent nonspecific protein binding. These coated latex particles were then used in an egglutination assay for the detection of HCV antibodies. The specimens were mixed in a ratio of 1:1 with the latex solution (0.5%). The agglutination pattern was complete in a period of 15 min. Assay results were read by eye and by microscopic examination. The results of serial dilution samples from a well characterized anti-HCV positive plasma sample are summarized in Table 7. A coating concentration of 0.3 mg/mL was found to give optimal sensitivity for antibody detection. As a control for specificity, pooled plasma specimens from normal donors were tested in the peptide VIII-BSA conjugate latex assay and were

found clearly negative.

Table 7

Rapid Detection of HCV Antibodies using VIIIE-BSA Sensitized Latex Particles and Scoring for Visual Agglutination Pattern

HCV Positive Control Dilution	Degree of Agglutination: VIIIE-BSA Latex Particle Coating Concentration 2.4 mg/mL	1.2 mg/mL	0.6 mg/mL	0.3 mg/mL
1:1	4+	4+	4+	4+
1:2	4+	4+	4+	4+
1:5	4+	4+	4+	4+
1:10	4+	4+	4+	4+
1:20	3+	4+	4+	4+
1:40	2+	3+	4+	4+
1:80	+/-	-	+	3+
1:160	-	-	-	+
1:320	-	-	-	+/-
1:640	-	-	-	+/-
NP 1:1	-	-	-	-

NP: Pooled Normal Plasma

EXAMPLE 8

SYNTHESIS OF OCTAMERIC HCV PEPTIDE ANTIGENS AS KEY COMPONENTS OF IMMUNOGENS/VACCINES

The use of a limited sequential propagation of a trifunctional amino acid (or similar analogues) to form a core that serves as a low molecular weight matrix is the basic underlying principle for the formation of a radially branching multimeric peptide antigen system. The trifunctional amino acid, Boc-Lys(Boc), or di-(Boc)-Lys is most suitable since both N^{α}- and N^{ϵ}- emino acid groups are available as reactive ends. Thus, sequential propagation of di-(Boc)-Lys will generate 2^n reactive ends. For example, the first level coupling of di-(Boc)-Lys will produce two reactive amino ends as a bivalent peptide antigen. Sequential generations of a second, third, and fourth step with di-(Boc)-Lys will therefore generate tetravalent, octavalent, and hexadecavalent peptide antigens respectively. As an example, an octameric HCV peptide immunogen with a structure of [Gln-Gly-Trp-
Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-
Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-
Val-Cys-Gly-Pro-Val-Tyr-Cys]$_8$-Lys$_4$-Lys$_2$-Lys was synthesized as a prototype immunogen used in our immunization of guinea pigs. This octameric antigen contains a small heptalysyl core (<20%) and the bulk (>80%) is formed by a high density of uniform peptide-antigen layered around the core matrix. This design differs from the conventional peptide-carrier conjugate which contains a large protein carrier such as PPD or

KLH and a low density of peptide antigens randomly distributed on the protein carrier surface in an unidentified form.

For the synthesis of octameric HCV peptide immunogen, a combination of Boc-amino acid resin-bound benzhydrylamide and tBoc-chemistry was used. An octameric heptalysyl core resin was prepared by coupling di-t-Boc Lys onto an extra low loading 0.14 mmole/g MBHA (4-methyl benzhydrylamine) resin on a Biosearch 9500 instrument. During each of the two coupling cycles, di-(Boc)-Lys was used for single coupling followed by two capping reactions (with 0.3 M acetylimidazole in DMF dimethylformamide).

After two additional di-(Boc)-Lys couplings onto the first di-(NH_2) Lys-resin, the substitution level of synthetic octameric resin was determined by ninhydrin test and found to have an eppropriate level of -NH_2 groups, as calculated based on a theoretical coupling yield, and was used thereafter for the synthesis of octameric peptide antigens each with a predefined amino acid sequence according to the standard t-Boc chemistry.

Acid-lebile tert-butyloxycarbonyl (t-Boc) was used for the protection of N-α amino acid. The following functional side-chain protecting groups were used: O-benzyl for Thr, Ser, Glu and Tyr; N^{δ}-tosyl for Arg; BOM(i.e. Boc-N^{im}-Benzyloxymethyl-) for His; N'-dichlorobenzyloxycarbonyl for Lys; S-4-methylbenzyl- for Cys; O-cyclohexyl for Asp and CHO for Trp. Successive amino acids were added as dictated by the sequence. The resultant octameric peptidyl resin was cleaved by anhydrous HF [0°C for 1 hr in the presence of 10% (v/v) anisole]. The released octameric antigen was extracted by acetic acid, after two cycles of ether washings of the cleaved peptidyl resin, and lyophilized to dryness so as to be ready for use as an immunogen. A computer-generated picture of such an octameric immunogen is shown in Fig. 1.

Example 9

Relative (%) Immunoreactivity for Envelope/NS-1 Protein Derived Synthetic Peptides by an Enzyme-linked Immunosorbent Assay

Wells ot 96-well plates were coated for 1 hour at 37° C with each ot the 21 peptides (designated as 255 A-C; 244 A,B; 254 A-C; 248 A-C; 247 A-E and 246 A-E, synthesized with sequences derived from the envelope/NS-1 region of HCV, at 5 ug/mL at 100 uL per well in 10 mM $NaHCO_3$ buffer, pH 9.5. The immunoreactivity ot each peptide was measured by an 8 member HCV serum panel (Panel I). All 21 peptides were lacking in immunoreactivity on this standard screening HCV panel. However, peptide 254B was found to have some weak reactivity with one panel member, and upon further testing it also reacted strongly with a sample derived from an anti-HCV positive (positive with peptides VIIIE and IIH) plasmapheresis donor with elevated (100 i.u./L) alanine aminotransferase (ALT) enzyme activity. To select a panel of samples with reactivity to peptides from the envelope/NS-1 region, 97 such samples from anti-HCV positive plasmapheresis donors with elevated ALT levels were tested with peptide 254B. One sample had an absorbance of 3.214, and a second sample, 2.184. 17 samples with the greatest reactivity with peptide 254B were chosen to form a third panel (Panel III) to screen for the immunoreactivity of the other 20 peptides from the envelope/NS-1 region. The relative (%) immunoreactivity, using peptide 254B as a standard, is given in Table 8a. The individual absorbance values of each ot the 17 samples on the four peptides with the greatest reactivity, i.e. 255C (pep7), 254B (pep8), 247B (pep9), and 246D (pep10), are listed in Table 8b.

Since a unique immunoreactivity pattern with panel III members is observed for each of the four peptides (see the boxed value), all four peptides or their analogues are therefore found to be useful as antigens for the development of immunoassays designed for the detection and screening for antibodies to HCV, particularly to the envelope/NS-1 associated proteins. This "unique" yet "complementary" immunoreactivity pattern conferred by the tour peptides as illustrated in Table 8b further demonstretes that the utility of the peptides as entigens for HCV antibody detection, as immunogens for the development of antibodies to HCV envelope/NS-1 protein, and as vaccines for the protection of HCV infection.

Since all four peptides (pep7, pep8, pep9, and pep10) are derived from the variable regions of the HCV envelope/NS-1 proteins, examples of substitution analogues for these four peptides are given in Table 8c based on the amino acid sequence (single letter code) information derived from three different HCV strains.

In addition to screening on Panel III samples, the envelope/NS-1 peptides were also tested against samples from plasmapheresis donors who had eleveted ALT levels but were nonreactive on the HCV trom the core (e.g. peptide VIIIE) and NS-4 (e.g. peptide IIH) regions screening EIA. Six of these samples, which may represent early seroconversion samples, were reactive on one or more envelope/NS-1 peptide (Table 8d). The absorbance values on these HCV EIA nonreactive samples are lower than the values found for Panel III samples. In the case of Pep7 and Pep10, their shorter segments, 225B and 246C, respectively,

gave greater immunoreactivity, in contrast to the performance on Panel III.

Table 8a

Synthetic Peptides with their Amino Acid Sequences Derived from the HCV Envelope /NS-1 Protein Region

		% Relative Immunoreactivity
	GLTYPASATQVRNSTGLYHVTNDCPNSSIVYEAHDAILHTPGCVPCVREGNVSRCWVAMTPTVATRDGKLPATQLRRHIDLLVGSATLC	
255A	TNDCPNSSIVYEAHDAILHTPGCVPCVREGNVSRC	9.8
255B	VRNSTGLYHVTNDCPNSSIVYEAHDAILHTPGCVPCVREGNVSRC	18.7
255C(Pep7)	GLTVRASATQVRNSTGLYHVTNDCPHSSIVYEAHDAILHTPGGVRGVREGNVSRC	51.4
244A	CWVAMTPTVATRDGKLPATQLRRHIDLLVGSATLG	3.8
244B	CVREGNVSRCWVAMTPTVATRDGKLPATQLRRHIDLLVGSATLG	5.8
	SALTVQDLGGSVFLIGQLFTFSPRRHWTTQGCNCSIYPGHITGHRMAWDMMMNWSPTA	
254A	TQGCNCSIYPGHITGHRMAWDMMMNWSPTA	21.4
254B(Pep8)	FTFSPRRHWTTQGGNCSIYPGHITGHRMAWDMMMNWSPTA	100.0
254C	GGSVFLIGQLFTFSPRRHWTTQGCNCSIYPGHITGHRMAWDMMMNWSPTA	16.7
	ALVMAGLLRIPQAILDMIAGAHWGVLAGIAYFSMVGNWAKVLVVLLLFAGVDAETIVSGQAARAMSGLVSLFTPGAKQNIQLINTNGSWHINSTALNCNESLNTGWLAGLIYQHKFNSSGCPERLASC	
248A	DNIAGAHWGVLAGIAYFSMVGNWAK	3.1
248B	QLLRIPQAILDNIAGAHWGVLAGIAYFSMVGNWAK	2.5
248C	ALVMAGLLRIPQAILDNIAGAHWGVLAGIAYFSMVGNWAK	5.3
247A	QAARAMSGLVSLFTPGAKQNIQLIN	39.0
247B(Pep9)	VDAETIVSGQAARAMSGLVSLFTPGAKQNIQLIN	41.3
247C	VLVVLLLFAGVDAETIVSGQAARAMSGLVSLFTPGAKQNIQLIN	21.9
247D	YFSMVGNWAKVLVVLLLFAGVDAETIVSGQAARAMSGLVSLFTPGAKQNIQLIN	22.7
247E	LAGIAYFSMVGNWAKVLVVLLLFAGVDAETIVSGQAARAMSGLVSLFTPGAKQNIQLIN	26.5
246A	TGWLAGLIYQHKFNSSGCPERLASC	37.5
246B	CNESLNTGWLAGLIYQHKFNSSGCPERLASC	19.8
246C	INSTALNCNESLNTGWLAGLIYQHKFNSSGCPERLASC	97.6
246D(Pep10)	VNINSTALNCNESLNTGWLAGLIYQHKFNSSGCPERLASC	98.6
246E	KQNIQLINTNGSWINSTALNCNESLNTGWLAGLIYQHKFNSSGCPERLASC	10.5

Table 8b

Absorbance of Envelope/NS-1 Peptides on Selected Anti-HCV Positive Samples with Elevated ALT Levels

Sample	Pep7	Pep8	Pep9	Pep10
1	**1.520**	0.475	0.335	1.085
2	0.017	**0.612**	0.009	0.068
3	0.235	**0.774**	0.341	0.090
4	0.066	0.279	0.268	**1.038**
5	0.711	0.076	**1.412**	0.077
6	0.106	0.058	0.027	**1.428**
7	0.784	**2.184**	0.241	**3.468**
8	0.037	0.120	0.055	**2.992**
9	0.019	**1.597**	0.177	0.334
10	0.313	**3.214**	**2.564**	1.488
11	0.035	0.025	**0.763**	0.045
12	**2.132**	1.497	0.160	0.408
13	**2.266**	1.573	0.129	0.451
14	0.047	**1.155**	0.170	0.037
15	0.012	0.053	0.030	**2.280**
16	0.064	**2.200**	0.039	0.810
17	0.077	**0.541**	0.069	0.111

Table 8c

PEP7 (255C)	(J-1)	CLTVPASAYQVRNSTGLYHVTHDCPNSSIVYEANDAILHTPGCVPCVREGNVSRC
	(J-4)	CLTIPASAYEVRNVSGIYHVTHDCSNSSIVYEAADNINHTPGCVPCVREDWSSRC
	(HCV-J)	CLTIPASAYEVRNVSGIYHVTHDCSNSSIVTEAADWIWHTPGCVPCVRESNFSRC
PEP8 (2548)	(J-1)	FTFSPRRNVTTQGCNCSITPGHITGNRNAWDMNMNWSPTIA
	(J-4)	FTFSPRRNETVQDCHCSITPGHLSGNRNAWDNNMNWSPTTT
	(HCV-J)	FTFSPRRTETVQDCNCSIYPGHVSGNRNAWDNNMNWSPTTT
PEP9 (2478)	(J-1)	VDAETIVSGGQAARAHSGLVSLFTPGAKQKIQLIN
	(J-4)	VDAETTTSGGAASNTTSTLASLFSPGASQRIQLVN
	(HCV-J)	VDGNTHVTGGRVASSTQSLVSWLSTQGPSQKIQLVN
PEP10 (2460)	(J-1)	WHINSTALNCNESLNTGWLAGLIYQHKFNSSGCPERLASC
	(J-4)	WHINRTALNCMDSLKTGFLAALFTTNRFNSSGCPERNASC
	(HCV-J)	WHINRTALNCMDSLQTGFIAALF-ANRFNASGCPERNASC

Examples of substitution analogues of pep7, pep8, pep9 and pep10 are given above based on the amino acid sequence (single letters code) information derived from three representative HCV strains (J-1, J-4 and J). The shared amino acid residues are boxed for purpose of comparison.

Table 8d

Absorbance of Envelope/NS-1 Peptides on Selected Samples Nonreactive on HCV Core (VIIIE) and NS-4(IIH) Peptides

Sample	255B	255C (Pep7)	254B (Pep8)	246C	246D (Pep10)
1	0.344	0.098	0.173	0.240	0.068
2	0.419	0.346	0.015	0.015	0.028
3	0.403	0.300	0.0111	0.023	0.029
4	0.021	0.021	0.222	0.046	0.049
5	0.300	0.231	0.014	0.009	0.009
6	0.012	0.017	0.044	0.402	0.102

EXAMPLE 10

SYNTHESIS OF OCTAMERIC HCV ENVELOPE/NS-1 PEPTIDE ANTIGENS AS KEY COMPONENTS OF IMMUNOGENS/VACCINES

Four octameric HCV envelope/NS-1 peptide immunogens with a structure of

[Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys]$_8$Lys$_4$Lys$_2$Lys (octameric pep7); [Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala]$_8$ Lys$_4$Lys$_2$Lys (octameric pep8); [Val-Asp-Ala-Glu-Thr-Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn]$_8$Lys$_4$Lys$_2$Lys (octameric pep9) and [Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Gln-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys]$_8$Lys$_4$Lys$_2$Lys (octameric pep10),

are synthesized respectively according to a general chemical synthesis procedure described in Example 8 and used as immunogens in our immunization of guinea pigs and chimpanzees.

These octameric peptides are injected as a mixture into healthy, naive animals both intradermally and subcutaneously at a dosage of 25 ug per mixture per kg body weight using 2% alum as an adjuvant. After the initial immunization, these animals are boosted at the same dose once per month for a period of four months. The animals are bled monthly and the collected immune sera are monitored for their anti-HCV envelope/NS-1 immunoreactivity. Six months after the last boost, the immunized chimpanzees are subsequently challenged by experimental inoculation with various dosages (e.g. 50 mL) of a proven infectious Factor VIII concentrate known to contain HCV so as to evaluate the efficacy in using a mixture of these octameric envelope/NS-1 peptides as a vaccine for the prevention of HCV infection.

EXAMPLE 11

Detection of Antibodies to HCV by a Peptide Based Enzyme Immunoassay (EIA) Using Format C

A total of 221 well-characterized clinical specimens categorized into four groups, (a) to (d), were tested on a representative HCV peptide based EIA with the plates coated with a mixture of peptides IIH, V and VIIIE at 5, 3 and 2 ug/mL respectively at 100 uL per well (Format C), containing both the HCV core and nonstructural peptides as shown in Table B.

Table B

Clinical Group	n	% positive for HCV antibodies
(a) AIDS/ARC patients	63	55.6
(b) HBsAg positive individuals	50	42.0
(c) HBc antibody positive antibodies	22	22.7
(d) Individuals with elevated (>100 i.u./L) alanine amino transferase (ALT) enzyme activity	86	91.5

EXAMPLE 12

Detection of Antibodies to HCV by Peptide Based HCV EIA Using Formats 1 to 6

The following five groups of serum specimens:

(a) Plasmapheresis donors with elevated (>100 i.u./L) alanine aminotransferase (ALT) enzyme activity (n=30);

(b) Blood donors with elevated (>45 i.u./L) ALT enzyme activity (n=15);

(c) Chronic NANBH patients (n=30);

(d) Other viral infections (n=11);

(e) Autoimmune disease patients (n=9);

were analyzed on representative HCV peptide based EIA kits according to the present invention, with the plates coated at 100 uL per well either with:

(i) Format 1: peptides VIII E, II H and pep11 at 0.5, 3 end 1 µg/mL each;

(ii) Format 2: peptides VIII E and pep11 at 0.5 and 1 µg/mL each;

(iii) Format 3: peptides VIII E, Pep11 and pep8 at 0.5, 1 and 10 µg/mL each;

(iv) Format 4: peptides VIII E and pep8 at 0.5 and 10 µg/mL each;

(v) Format 5: peptides VIII E, pep11 and pep12 at 0.5, 1 and 2 µg/mL each;

(vi) or Format 6: peptides VIII E end pep12 at 0.5 end 2 µg/mL each.

These kits represent core, NS-4 and NS-5 (Format 1), core and NS-5 (Formats 2, 5 and 6), core, NS-5 and env (Format 3) and core and env (Format 4).

The results of testing these 95 well characterized samples on Formats 1 through 6 are presented in Table 9. The results indicate that (30/30) of the samples in group (a) were reactive by Formats 1, 2 and 3; 90% (27/30) reactive by Format 4 and 97% (29/30) reactive by Formats 5 and 6. All samples in groups (b) and (c) were positive on all 6 formats. Groups (a), (b) end (c) were shown to be reactive by Format C described in Example 11.

Three samples in group (d) were reactive by Formats 1 to 4. In contrast, these samples were indicated as negative by Format C. Serum samples "86" and "124" apparently responded to the presence of pep11, and serum sample "VZV2500" was indicated as positive by the presence of pep8 in Formats 4 and 5.

All serum samples in group (c) were negative on all formats, including Format C.

Table 9

Antibody to HCV Detected By Peptide Based EIA Kits
(Absorbance 492nm)

Sample ID		Format 1	Format 2	Format 3	Format 4	Format 5	Format 6
NRC		0.065	0.075	0.056	0.061	0.060	0.019
WRC		0.650	0.454	0.953	0.967	0.403	0.340
SRC		2.183	1.791	2.580	2.635	1.589	1.331
a. Plasmapheresis, ALT >100 i.u./L							
1	-13	3.166	3.419	3.255	3.371	3.291	3.255
	-27	1.555	1.548	1.980	2.904	1.152	0.881
	-31	3.479	3.144	3.220	2.332	3.319	2.665
	-32	3.001	3.035	3.112	2.691	3.076	2.986
	-39	3.063	3.041	3.361	2.886	3.190	3.038
	-42	3.198	3.201	3.050	3.227	3.230	3.118
	-47	3.479	3.110	3.251	3.201	3.229	3.068
	-48	3.142	2.795	3.116	2.934	3.076	2.725
	-49	3.417	3.291	3.525	3.451	3.195	3.592
	-52	3.263	3.329	3.202	0.120	3.262	3.453
	-53	3.225	3.145	3.096	0.062	3.358	3.097
	-54	3.271	3.018	3.267	0.153	3.073	3.211
2	-4	1.012	0.881	1.542	1.767	0.807	0.745
	-6	3.229	2.964	3.169	3.052	3.076	2.897
	-9	2.691	2.416	2.766	2.967	2.119	1.844
	-26	3.222	3.055	3.095	3.167	3.195	2.951
	-32	3.226	3.372	3.368	3.194	3.496	3.417
	-33	3.151	2.918	3.147	3.027	3.108	3.129
	-34	3.059	3.021	3.143	3.167	3.145	3.320
	-38	3.241	3.116	2.967	3.055	3.213	3.137
	-41	2.964	2.593	2.841	2.964	2.469	2.252
	-43	3.146	2.092	2.541	2.627	1.999	1.920
	-46	2.927	2.818	2.998	2.983	2.556	2.415
	-58	3.285	3.444	3.218	3.191	3.355	3.095
	-60	3.094	2.975	3.113	3.167	2.683	2.640
	-61	2.784	2.345	2.501	2.751	2.007	2.212
	-62	3.320	3.076	3.095	3.076	3.003	2.787
	-77	0.815	0.682	1.096	0.418	0.164	0.152
	-82	3.020	2.982	1.826	3.001	3.032	2.820
	-83	3.076	2.914	3.049	2.996	2.928	2.808
b. Elevated ALT blood donors (ALT > i.u./L)							
ALT	-1	3.017	3.035	3.116	3.165	3.167	2.920
	-2	3.256	3.166	3.165	2.974	3.292	3.091
	-3	3.153	3.328	3.291	3.105	3.203	3.230
	-4	2.969	2.894	3.096	3.144	2.880	2.866
	-5	3.073	2.956	2.968	2.952	3.376	2.985
	-7	3.218	3.020	3.157	2.980	2.951	3.060
	-8	3.074	2.930	3.094	3.197	3.121	3.012
	-10	3.479	3.228	3.226	3.109	3.432	3.952
	-11	3.398	3.283	3.140	3.035	3.285	3.222
	-53	3.330	3.029	3.253	3.290	2.974	3.070
	-56	3.151	3.086	3.176	3.202	3.107	3.085
	-69	3.021	3.170	3.167	3.318	3.019	2.831
	-70	3.074	3.035	2.951	3.073	3.054	3.184
	-71	2.985	2.901	3.080	3.039	2.902	2.900
	-82	3.230	3.120	3.085	2.977	3.298	3.096

c. Chronic NANBH							
N	-2	3.320	3.052	2.981	3.283	3.032	2.999
	-3	3.285	3.036	3.095	3.167	3.077	3.094
	-4	3.117	3.469	3.590	3.291	2.259	3.141
	-7	3.027	3.008	3.061	3.065	2.962	2.806
	-8	3.285	3.146	3.117	3.194	3.122	3.195
	-9	2.886	3.001	3.072	2.985	2.848	2.859
	-10	2.606	2.268	2.027	0.423	2.338	1.104
	-14	3.054	2.808	2.856	2.995	2.341	2.041
	-23	3.228	3.050	3.067	3.225	3.152	3.109
	-25	3.891	2.462	3.190	3.165	1.982	2.091
	-27	3.194	2.926	3.165	3.029	3.143	3.168
	-28	3.027	3.106	3.259	3.175	3.176	3.202
	-34	3.057	3.037	3.035	3.144	2.907	2.892
	-36	3.304	3.213	3.000	3.033	3.075	3.115
	-41	3.217	3.283	3.039	3.248	3.290	3.249
	-42	2.997	2.858	3.196	3.094	3.097	2.805
	-44	3.391	3.477	3.350	3.254	3.353	3.387
	-45	3.318	3.096	2.964	3.250	3.319	3.036
	-49	3.292	3.371	3.416	3.255	3.292	3.370
	-54	3.329	3.294	3.105	3.105	3.177	3.203
	-57	3.197	3.169	3.221	3.141	3.120	3.018
	-60	3.115	3.035	3.090	3.072	3.096	2.873
	-65	2.020	1.816	1.898	2.376	1.133	1.284
	-67	2.265	1.776	2.356	2.396	1.319	0.911
	-68	3.178	3.177	3.200	3.176	3.530	3.087
	-69	3.222	3.167	3.165	3.283	3.399	3.097
	-77	1.438	1.346	2.548	2.397	1.055	1.071
	-78	2.457	2.038	2.251	2.300	1.642	1.494
	-79	3.225	3.197	3.076	3.142	3.224	3.169
	-80	3.138	3.074	3.135	3.054	3.137	2.896
d. Other Viral Infections							
HAV	-86	0.558	0.316	0.607	0.054	0.037	0.014
	-88	0.018	0.021	0.062	0.054	0.014	0.018
	-92	0.045	0.061	0.058	0.050	0.043	0.007
	-120	0.057	0.076	0.051	0.032	0.051	0.026
	-121	0.052	0.138	0.094	0.065	0.072	0.026
	-124	0.816	1.178	0.622	0.062	1.082	0.017
	-125	0.014	0.016	0.050	0.031	0.012	0.010
	-126	0.105	0.134	0.109	0.081	0.117	0.068
EBV	-2331	0.021	0.021	0.023	0.020	0.012	0.012
VZV-M002		0.035	0.030	0.154	0.108	0.025	0.012
VZV	-2500	0.090	0.138	0.976	0.923	0.084	0.032
e. Autoimmune							
	-209	0.102	0.079	0.117	0.097	0.066	0.028
	-210	0.002	0.003	0.018	0.011	0.002	0.005
	-211	0.016	0.019	0.134	0.168	0.022	0.016
	-212	0.016	0.020	0.075	0.080	0.019	0.006
	-213	0.008	0.009	0.055	0.076	0.005	0.002
	-215	0.118	0.095	0.226	0.282	0.093	0.060
	-216	0.039	0.037	0.100	0.105	0.042	0.022
	-217	0.019	0.021	0.068	0.056	0.023	0.012
	-218	0.032	0.022	0.110	0.086	0.059	0.031

EXAMPLE 13

Comparison of Test Results Using the Six Peptide Based HCV EIA Formats (1-6) on Random Blood Donors

Random blood donor samples (n = 100) were tested by Formats 1 to 6. All 100 samples were negative on Formats 2, 5 and 6. Sample 14 had an absorbance of 0.680 on Format 1, and sample 34 had an absorbance of 0.601 and 0.551 on Formats 3 and 4, respectively. For the calculation of mean absorbance and standard deviation, absorbance values >0.500 were omitted from anelysis. Table 10 lists the mean absorbance and standard deviation of the 100 samples on Formats 1-6.

Table 10

Mean Absorbance (A492nm) ± SD of 100 Random Blood Donors

	Format 1	Format 2	Format 3	Format 4	Format 5	Format 6
Mean	0.040	0.035	0.068	0.061	0.030	0.017
S.D.	0.036	0.029	0.046	0.046	0.039	0.032

EXAMPLE 14

Peptide Analogues from HCV Variant Strains for Subtyping HCV-Reactive Sera

Immunoreactive peptides pep7, pep8, pep9 and pep19 derived from the ENV and NS-1 regions, and their analogues with sequences taken from HCV strains HC-J1, CDC/HCV 1, H, HC-J4, HCV-JH, HCV-J, BK, HC-J6 and HC-J7 are synthesized to have the amino acid sequences according to Table 11. The immunoreactive peptides are coated at 5 µg/mL at 100 uL per well in wells of microtiter plates and are used to assay HCV positive sera from Taiwan, Japan, Europe, Austrelia end North America to classify their HCV reactivity into subtypes e.g., HCV-J1, HC-J4, HC-J6 and HC-J7. These peptides derived from hypervariable regions of HCV are useful to distinguish the subtypes of HCV responsible for the infection.

Table 11

Immunoreactive Pep7, Pep8, Pep9 and Pep19 and Their Substitution Analogues Derived from the HCV ENV/NS-1 Regions

(Pep7, 255C, aa 184-236)

```
HC-J1     CLTVPASAYQVRNSTGLYHVTNDCPNSSIVYEAHDAILHTPGCVPCVREGNVSRC
HCV1      ------------------------------A----------------A---
HCV-H     ----------S-----------------A----------------A---
HC-J4     ---I-----E---VS-I-------S--------A-M-M----------D-S---
HCV-JH    ---I-----E---VS-I-------S--------A-V-M-A---------N-S---
HCV-J     ---I-----E---VS-I-------S--------A-M-M----------S-F---
HCV-BK    ---T-----E-H-VS-I-------S-A------A-L-M----------S---
HCV-J6    -I-T-V--AE-K-ISTG-M-----T-D--TWQLQA-V--V------EKV--T---
HCV-J7    -V---V--VE---ISSS-YA----S-N--TWQLTN-V--L------ENDNGTL--
```

(Pep8, 254B, aa 291-330)

```
HC-J1     FTFSPRRHWTTQGCNCSIYPGHITGHRMAWDMMMNWSPTA
HCV1      ----------------------------------------T
HCV-H     ----------D-----------------------------
HC-J4     --------E-V-D---------LS----------------T
HCV-JH    --------E-V-D---------VS----------------
HCV-J     --------YE-V-D---------VS----------------T
HCV-BK    --------V-L-D---------VS----------------T
HCV-J6    -IV--QH--FV-D--------T-------------------
HCV-J7    -II--E--NF--E------Q----------L-----L
```

(Pep9, 247B, aa 381-415)

```
HC-J1     VDAETIVSGGQAARAMSGLVSLFTPGAKQNIQLIN
HCV-J     --GH-H-T--RV-SSTQS---WLSQ-PS-K---V-
HCV-BK    --GD-H-T--AQ-KTTNR---M-AS-PS-K-----
```

(Peptide 19, 244B, aa 229-272)

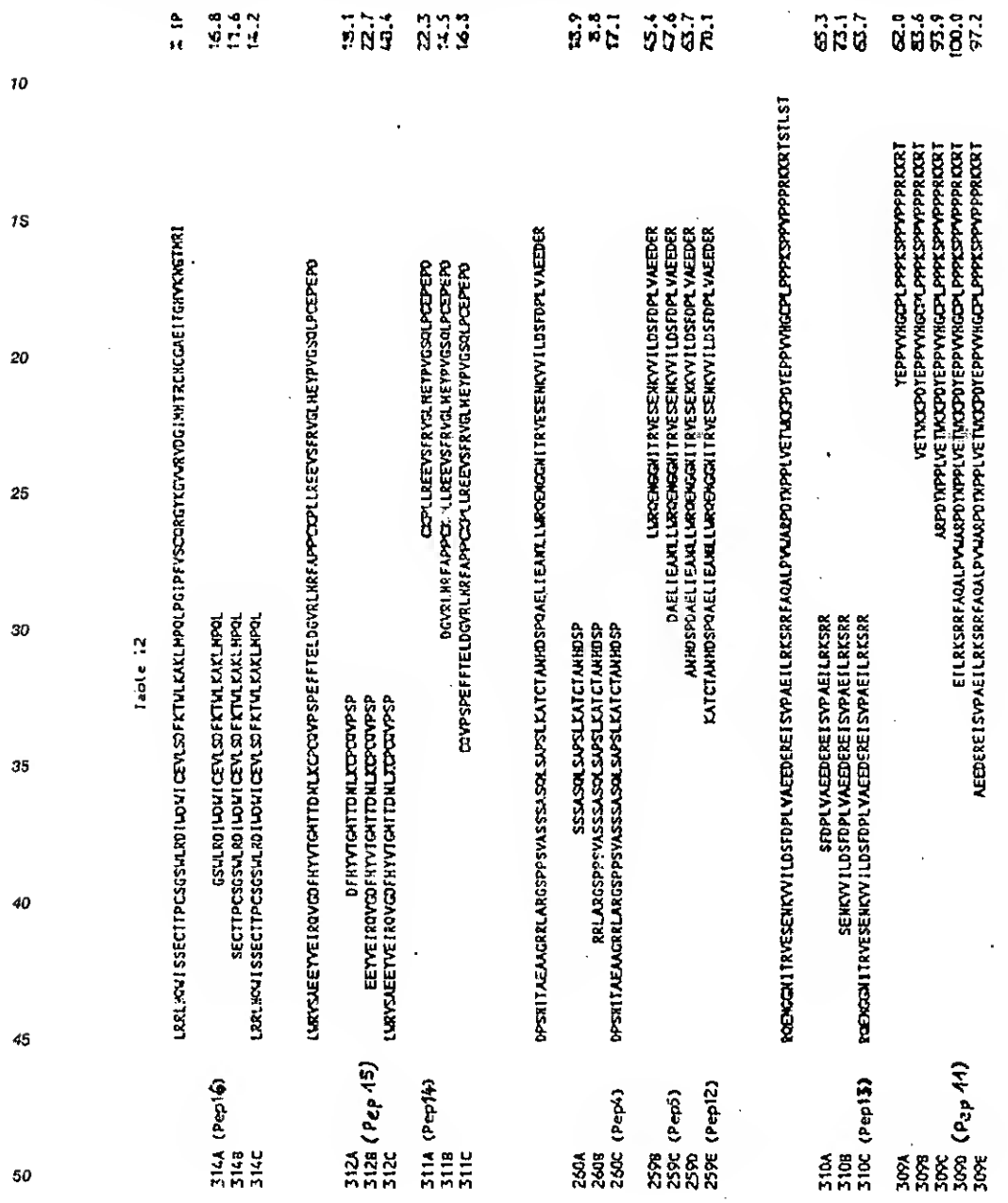
```
HC-J1     CVREGNVSRCWVAMTPTVATRDGKLPATQLRRHIDLLVGSATLC
HCV1      ------A-------------------------------------
HCV-H     ------A------V----------T-----------------
HC-J4     ----D-S------L---L-A-NASV-T-TI---V-----A-AF-
HCV-JH    ----N-S------L---L-A-NASV-T-T----V-----T-AF-
HCV-J     ----S-F------L---L-A-NSSI-T-TI---V-----A-A--
HCV-BK    ------S------L---L-A-NVTI-T-TI---V-----A-AF-
HCV-J6    -EKV--T----IPVS-N--VQQPGALTQG--T---MV-M-----
HCV-J7    -ENDNGTL---IQV--N--VKHRGALTHN--T-V-MI-MA--V-
```

EXAMPLE 15

Comparison of Immunoreactivity for NS-5 Protein Derived Synthetic Peptides

Wells of 96-well plates were coated for 1 hour at 37° C with each of the 23 peptides (designated as 259A-259E, 260A-260C, 309A-309C, 310A-310C, 311A-311C, 312A-312C and 314A-314C) synthesized with sequences derived from the NS-5 region, at 5 µg/mL at 100 µL per well in 10 mM $NaHCO_3$ buffer, pH 9.5.

The immunoreactivity of each peptide was measured by an 8 member HCV serum panel (Panel I). The peptide with the greatest immunoreactivity was pep11, designated 309C in Table 12. When the immunoreactivity of pep11 was used as a standard to calculate the relative immunopotency for the other NS-5 peptides, the peptides in series 309-314 were seen to be equal to or more reactive than pep4 and pep5 from Example 4. The extension of pep5 to include an additional 10 residues (259E, i.e. pep12) increased the relative immunopotency from 47.6% to 70.1%.



EXAMPLE 16

Immunoreactivity of a NS-2 Protein-derived Synthetic Peptide

Wells of 96-well plates were coated for 1 hour at 37° C with 9 synthetic peptides derived from the NS-2

region of HCV. The results (Table 13) show that peptide 2898 (i.e. pep17) was immunoreactive with selected anti-HCV positive samples with elevated ALT levels.

Table 13

Absorbance of NS-2 Peptides on Selected Anti-HCV Positive Samples with Elevated ALT Levels

Sample	289B
1	0.263
4	0.311
7	0.266
18	0.751

EXAMPLE 17

Immunoreactivity of NS-3 Protein-derived Synthetic Peptide with Sera from Individuals with Early HCV Infection

Wells of 96-well plates were coated for 1 hour at 37°C with synthetic peptide 315D (i.e. pep18) derived from the NS-3 region of HCV. The results (Table 14) show that peptide 315D was strongly reactive with two serial samples from a plasmapheresis donor with elevated ALT levels.

Table 14

Absorbance of NS-3 Peptide on Serial Samples from Plasmapheresis Donor with Elevated ALT Levels

Sample	315D
A	1.983
B	1.890

EXAMPLE 18

Detection of Antibodies to HCV NS-1 and ENV Regions by Peptide Based EIA Using Formats 7 and 8

Plasmapheresis samples with elevated ALT levels were analyzed on representative HCV peptide based EIAs according to the present invention with plates coated either with (i) pep1 and pep10C at 10 and 10 µg/mL each (Format 7, NS-1 kit) or (ii) pep7 and pep8 at 10 and 10 µg/mL each (Format 8, ENV kit). The results on HCV positive samples with elevated ALT levels are shown in Table 15, indicating a subpopulation of HCV infected individuals develop specific humoral immune responses directed at unique regions of the NS-1 and ENV proteins.

Table 16

Absorbance (492nm) of Selected Samples with Elevated ALT Levels on Formats 7 and 8

Sample	Format 7 NS-1	Format 8 ENV
1	0.804	1.499
2	0.707	2.487
3	0.441	1.649
4	2.651	2.868
5	0.064	1.569
6	0.244	0.790
7	0.382	0.692
8	1.438	1.226
9	0.304	0.411
10	0.160	0.282
11	0.079	0.599
12	0.286	0.302
13	0.045	0.610
14	3.058	2.862

Cutoff OD_{492nm} = 0.200

EXAMPLE 19

Synthesis of Substitution Analogues of Octameric HCV Envelope Peptide Antigen as Components of HCV Immunogens/Vaccines

Substitution analogues of octameric HCV envelope pep7, pep8 and pep19 with a structure of:

(a) [Cys-Leu-Thr-Ile-Pro-Ala-Ser-Ala-Tyr-Glu-Val-Arg-Asn-Val-Ser-Gly-Ile-Tyr-Mis-Val-Thr-Asn-Asp-Cys-Ser-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-Ala-Asp-Val-Ile-Met-His-Ala-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Asn-Asn-Ser-Ser-Arg-Cys-]$_8K_4K_2K$ (an analogue of octameric pep7 with sequence taken from HCV-JH);

(b) [Cys-Ile-Thr-Thr-Pro-Val-Ser-Ala-Ala-Glu-Val-Lys-Asn-Ile-Ser-Thr-Gly-Tyr-Met-Val-Thr-Asn-Asp-Cys-Thr-Asn-Asp-Ser-Ile-Thr-Trp-Gln-Leu-Gln-Ala-Ala-Val-Leu-His-Val-Pro-Gly-Cys-Val-Pro-Cys-Glu-Lys-Val-Gly-Asn-Thr-Ser-Arg-Cys-]$_8K_4K_2K$ (an analogue of octameric pep7 with sequence taken from HCV-J6);

(c) [Cys-Val-Thr-Val-Pro-Val-Ser-Ala-Val-Glu-Val-Arg-Asn-Ile-Ser-Ser-Ser-Tyr-Tyr-Ala-Thr-Asn-Asp-Cys-Ser-Asn-Asn-Ser-Ile-Thr-Trp-Gln-Leu-Thr-Asn-Ala-Val-Leu-His-Leu-Pro-Gly-Cys-Val-Pro-Cys-Glu-Asn-Asp-Asn-Gly-Thr-Leu-Arg-Cys-]$_8K_4K_2K$ (an analogue of octameric pep7 with sequence taken from HCV-J6);

(d) [Phe-Thr-Phe-Ser-Pro-Arg-Arg-Mis-Glu-Thr-Val-Gln-Asp-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Val-Ser-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala-]$_8K_4K_2K$ (an analogue of octameric pep8 with sequence taken from HCV-JH);

(e) [Phe-Ile-Val-Ser-Pro-Gln-Mis-His-Mis-Phe-Val-Gln-Asp-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-Thr-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-

Ala-]$_8$K$_4$K$_2$K (an analogue of octameric pep8 with sequence taken from HCV-J6);

(f) [Phe-Ile-Ile-Ser-Pro-Glu-Arg-Asn-Phe-Thr-Gln-Glu-Cys-Asn-Cys-Ser-Ile-Tyr-Gln-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Leu-Asn-Trp-Ser-Pro-Thr-Leu-]$_8$K$_4$K$_2$K (an analogue of octameric pep8 with sequence taken from HCV-J7);

(g) [Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys-]$_8$K$_4$K$_2$K (Octameric pep19)

(h) [Cys-Val-Arg-Glu-Asn-Asn-Ser-Ser-Arg-Cys-Trp-Val-Ala-Leu-Thr-Pro-Thr-Leu-Ala-Ala-Arg-Asn-Ala-Ser-Val-Pro-Thr-Thr-Thr-Leu-Arg-Arg-His-Val-Asp-Leu-Leu-Val-Gly-Thr-Ala-Ala-Phe-Cys-]$_8$K$_4$K$_2$K (an analogue of octameric pep19 with sequence taken from HCV-JH);

(i) [Cys-Glu-Lys-Val-Gly-Asn-Thr-Ser-Arg-Cys-Trp-Ile-Pro-Val-Ser-Pro-Asn-Val-Ala-Val-Gln-Gln-Pro-Gly-Ala-Leu-Thr-Gln-Gly-Leu-Arg-Thr-His-Ile-Asp-Met-Val-Val-Met-Ser-Ala-Thr-Leu-Cys-]$_8$K$_4$K$_2$K (an analogue of octameric pep19 with sequence taken from HCV-J6);

(j) [Cys-Glu-Asn-Asp-Asn-Gly-Thr-Leu-Arg-Cys-Trp-Ile-Gln-Val-Thr-Pro-Asn-Val-Ala-Val-Lys-His-Arg-Gly-Ala-Leu-Thr-His-Asn-Leu-Arg-Thr-His-Val-Asp-Met-Ile-Val-Met-Ala-Ala-Thr-Val-Cys-]$_8$K$_4$K$_2$K (an analogue of octameric pep19 with sequence taken from HCV-J7);

respectively according to a general chemical synthesis procedure described in Example 7 and used as immunogens in our immunization of guinea pigs and chimpanzees.

These octameric peptides are injected as a mixture into healthy, naive animals both intradermally and subcutaneously at a dosage of 25 ug per mixture per kg body weight using 2% alum as an adjuvant. After the initial immunization, these animals are boosted at the same dose once per month for a period of four months. The animals are bled monthly and the collected immune sera are monitored for their anti-HCV envelope/NS-1 immunoreactivity. Six months after the last boost, the immunized chimpanzees are subsequently challenged by experimental inoculation with various dosages (e.g. 50 mL) of a proven infectious Factor VIII concentrate known to contain HCV so as to evaluate the efficacy in using a mixture of these

octameric envelope peptides as a vaccine for the prevention of HCV infection, initially by the evaluation of several serological/clinical markers, and subsequently, the observation of the appearance of clinical symptoms of NANBH in these animals.

The present invention has been illustrated in the ebove examples, which are not to be used to limit the scope of the invention.

SEQUENCE LISTING

SEQ ID No.: **241A**
Sequence Type: amino acid (AA)
Sequence Length: 37 AA

```
Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-
 5                  10                  15
Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-
            20                  25                  30
Val-Pro-Ala-Lys-Ser-Val-Cys
        35
```

SEQ ID No.: **241B**
Sequence Type: AA
Sequence Length: 45 AA

```
Cys-Arg-Pro-Leu-Thr-Asp-Phe-Asp-Gln-Gly-Trp-Gly-Pro-Ile-Ser-
 5                  10                  15
Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-
            20                  25                  30
Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-
        35                  40                  45
```

SEQ ID No.: **241C**
Sequence Type: AA
Sequence Length: 52 AA

```
Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys-Arg-Pro-Leu-Thr-Asp-Phe-Asp-
 5                  10                  15
Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-
            20                  25                  30
Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-
        35                  40                  45
Val-Pro-Ala-Lys-Ser-Val-Cys
    50
```

SEQ ID No.: **231A**
Sequence Type: AA
Sequence Length: 26 AA

```
Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-
 5                  10                  15
Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-Val-Tyr-Cys
            20                  25
```

SEQ ID No.: **231B**
Sequence Type: AA
Sequence Length: 34 AA

```
Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-
 5                  10                  15
Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-
            20                  25                  30
Pro-Val-Tyr-Cys
```

SEQ ID No.: **231C (Pep 1)**
Sequence Type: AA
Sequence Length: 42 AA

```
Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-
 5                  10                  15
Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-
            20                  25                  30
Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-Val-Tyr-Cys
            35                  40
```

SEQ ID No.: **231D**
Sequence Type: AA
Sequence Length: 50 AA

```
Cys-Arg-Pro-Leu-Thr-Asp-Phe-Asp-Gln-Gly-Trp-Gly-Pro-Ile-Ser-
                  5                  10                  15
Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-
                 20                  25                  30
Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-
                 35                  40                  45
Gly-Pro-Val-Tyr-Cys
                 50
```

SEQ ID No.: **231E**
Sequence Type: AA
Sequence Length: 57 AA

```
Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys-Arg-Pro-Leu-Thr-Asp-Phe-Asp-
                  5                  10                  15
Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-
                 20                  25                  30
Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-
                 35                  40                  45
Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-Val-Tyr-Cys
                 50                  55
```

SEQ ID No.: **232A (Pep 2)**
Sequence Type: AA
Sequence Length: 26 AA

```
Pro-Pro-Leu-Gly-Asn-Trp-Phe-Gly-Cys-Thr-Trp-Met-Asn-Ser-Thr-
                  5                  10                  15
Gly-Phe-Thr-Lys-Val-Cys-Gly-Ala-Pro-Pro-Cys
                 20                  25
```

SEQ ID No.: 232B
Sequence Type: AA
Sequence Length: 34 AA

Val-Phe-Val-Leu-Asn-Asn-Thr-Arg-Pro-Pro-Leu-Gly-Asn-Trp-Phe-
5 10 15
Gly-Cys-Thr-Trp-Met-Asn-Ser-Thr-Gly-Phe-Thr-Lys-Val-Cys-Gly-
20 25 30
Ala-Pro-Pro-Cys

SEQ ID No.: 232C
Sequence Type: AA
Sequence Length: 42 AA

Ser-Trp-Gly-Glu-Asn-Asp-Thr-Asp-Val-Phe-Val-Leu-Asn-Asn-Thr-
5 10 15
Arg-Pro-Pro-Leu-Gly-Asn-Trp-Phe-Gly-Cys-Thr-Trp-Met-Asn-Ser-
20 25 30
Thr-Gly-Phe-Thr-Lys-Val-Cys-Gly-Ala-Pro-Pro-Cys
35 40

SEQ ID No.: 232D
Sequence Type: AA
Sequence Length: 50 AA

Asp-Arg-Ser-Gly-Ala-Pro-Thr-Tyr-Ser-Trp-Gly-Glu-Asn-Asp-Thr-
5 10 15
Asp-Val-Phe-Val-Leu-Asn-Asn-Thr-Arg-Pro-Pro-Leu-Gly-Asn-Trp-
20 25 30
Phe-Gly-Cys-Thr-Trp-Met-Asn-Ser-Thr-Gly-Phe-Thr-Lys-Val-Cys-
35 40 45
Gly-Ala-Pro-Pro-Cys
50

SEQ ID No.: 233C
Sequence Type: AA
Sequence Length: 42 AA

Leu-His-Cys-Pro-Thr-Asp-Cys-Phe-Arg-Lys-His-Pro-Asp-Ala-Thr-
5 50 15
Tyr-Ser-Arg-Cys-Gly-Ser-Gly-Pro-Trp-Ile-Thr-Pro-Arg-Cys-Leu-
20 25 30
Val-Asp-Tyr-Pro-Tyr-Arg-Leu-Trp-His-Trp-Pro-Cys
35 40

SEQ ID No.: 234A
Sequence Type: AA
Sequence Length: 23 AA

Glu-Ala-Ala-Cys-Asn-Trp-Thr-Arg-Gly-Glu-Arg-Cys-Asp-Leu-Glu-
5 10 15
Asp-Arg-Asp-Arg-Ser-Glu-Leu-Ser
20

SEQ ID No.: 234B
Sequence Type: AA
Sequence Length: 31 AA

Val-Gly-Gly-Val-Glu-His-Arg-Leu-Glu-Ala-Ala-Cys-Asn-Trp-Thr-
5 10 15
Arg-Gly-Glu-Arg-Cys-Asp-Leu-Glu-Asp-Arg-Asp-Arg-Ser-Glu-Leu-
20 25 30
Ser

SEQ ID No.: 234C
Sequence Type: AA
Sequence Length: 39 AA

```
Thr-Ile-Phe-Lys-Ile-Arg-Met-Tyr-Val-Gly-Gly-Val-Glu-His-Arg-
 5                  10                  15
Leu-Glu-Ala-Ala-Cys-Asn-Trp-Thr-Arg-Gly-Glu-Arg-Cys-Asp-Leu-
            20                  25                  30
Glu-Asp-Arg-Asp-Arg-Ser-Glu-Leu-Ser
            35
```

SEQ ID No.: 272A
Sequence Type: AA
Sequence Length: 41 AA

```
Pro-Val-Val-Pro-Gln-Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-
 5                  10                  15
Thr-Gly-Ser-Gly-Lys-Ser-Thr-Lys-Val-Pro-Ala-Ala-Tyr-Ala-Ala-
            20                  25                  30
Gln-Gly-Tyr-Lys-Val-Leu-Val-Leu-Asn-Pro-Ser
            35                  40
```

SEQ ID No.: 272B
Sequence Type: AA
Sequence Length: 55 AA

```
Thr-Thr-Met-Arg-Ser-Pro-Val-Phe-Thr-Asp-Asn-Ser-Ser-Pro-Pro-
 5                  10                  15
Val-Val-Pro-Gln-Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-Thr-
            20                  25                  30
Gly-Ser-Gly-Lys-Ser-Thr-Lys-Val-Pro-Ala-Ala-Tyr-Ala-Ala-Gln-
            35                  40                  45
Gly-Tyr-Lys-Val-Leu-Val-Leu-Asn-Pro-Ser
            50                  55
```

SEQ ID No.: 272C
Sequence Type: AA
Sequence Length: 66 AA

```
Ala-Val-Asp-Phe-Ile-Pro-Val-Glu-Asn-Leu-Glu-Thr-Thr-Met-Arg-
                    5                  10                  15
Ser-Pro-Val-Phe-Thr-Asp-Asn-Ser-Ser-Pro-Pro-Val-Val-Pro-Gln-
                20                  25                  30
Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-Thr-Gly-Ser-Gly-Lys-
                35                  40                  45
Ser-Thr-Lys-Val-Pro-Ala-Ala-Tyr-Ala-Ala-Gln-Gly-Tyr-Lys-Val-
                50                  55                  60
Leu-Val-Leu-Asn-Pro-Ser
                65
```

SEQ ID No.: 278A
Sequence Type: AA
Sequence Length: 29 AA

```
Pro-Val-Val-Pro-Gln-Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-
                    5                  10                  15
Thr-Gly-Ser-Gly-Lys-Ser-Thr-Lys-Val-Pro-Ala-Ala-Tyr-Ala
                20                  25
```

SEQ ID No.: 278B
Sequence Type: AA
Sequence Length: 36 AA

```
Phe-Thr-Asp-Asn-Ser-Ser-Pro-Pro-Val-Val-Pro-Gln-Ser-Phe-Gln-
                    5                  10                  15
Val-Ala-His-Leu-His-Ala-Pro-Thr-Gly-Ser-Gly-Lys-Ser-Thr-Lys-
                20                  25                  30
Val-Pro-Ala-Ala-Tyr-Ala
                35
```

SEQ ID No.: 278C
Sequence Type: AA
Sequence Length: 48 AA

```
Val-Glu-Asn-Leu-Glu-Thr-Thr-Met-Arg-Ser-Pro-Val-Phe-Thr-Asp-
 5                  10                  15
Asn-Ser-Ser-Pro-Pro-Val-Val-Pro-Gln-Ser-Phe-Gln-Val-Ala-His-
            20                  25                  30
Leu-His-Ala-Pro-Thr-Gly-Ser-Gly-Lys-Ser-Thr-Lys-Val-Pro-Ala-
        35                  40                  45
Ala-Tyr-Ala
```

SEQ ID No.: 278D
Sequence Type: AA
Sequence Length: 54 AA

```
Ala-Val-Asp-Phe-Ile-Pro-Val-Glu-Asn-Leu-Glu-Thr-Thr-Met-Arg-
 5                  10                  15
Ser-Pro-Val-Phe-Thr-Asp-Asn-Ser-Ser-Pro-Pro-Val-Val-Pro-Gln-
            20                  25                  30
Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-Thr-Gly-Ser-Gly-Lys-
        35                  40                  45
Ser-Thr-Lys-Val-Pro-Ala-Ala-Tyr-Ala
    50
```

SEQ ID No.: 275A
Sequence Type: AA
Sequence Length: 38 AA

```
Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-
 5                  10                  15
Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-
            20                  25                  30
Ile-Ile-Cys-Asp-Glu-Cys-His-Ser
        35
```

SEQ ID No.: 275B
Sequence Type: AA
Sequence Length: 71 AA

```
Lys-Val-Leu-Val-Leu-Asn-Pro-Ser-Val-Ala-Ala-Thr-Leu-Gly-Phe-
 5                  10                  15
Gly-Ala-Tyr-Met-Ser-Lys-Ala-His-Gly-Ile-Asp-Pro-Asn-Ile-Arg-
            20                  25                  30
Thr-Gly-Val-Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-
        35                  40                  45
Thr-Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-
    50                  55                  60
Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser
65                  70
```

SEQ ID No.: 275C
Sequence Type: AA
Sequence Length: 94 AA

```
His-Leu-His-Ala-Pro-Thr-Gly-Ser-Gly-Lys-Ser-Thr-Lys-Val-Pro-
 5                  10                  15
Ala-Ala-Tyr-Ala-Ala-Gln-Gly-Tyr-Lys-Val-Leu-Val-Leu-Asn-Pro-
            20                  25                  30
Ser-Val-Ala-Ala-Thr-Leu-Gly-Phe-Gly-Ala-Tyr-Met-Ser-Lys-Ala-
        35                  40                  45
His-Gly-Ile-Asp-Pro-Asn-Ile-Arg-Thr-Gly-Val-Arg-Thr-Ile-Thr-
    50                  55                  60
Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-Ala-
65                  70                  75
Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-
            80                  85                  90
Glu-Cys-His-Ser
```

SEQ ID No.: 275D
Sequence Type: AA
Sequence Length: 117 AA

```
Thr-Met-Arg-Ser-Pro-Val-Phe-Thr-Asp-Asn-Ser-Ser-Pro-Pro-Val-
  5                  10                  15
Val-Pro-Gln-Ser-Phe-Gln-Val-Ala-His-Leu-His-Ala-Pro-Thr-Gly-
 20                  25                  30
Ser-Gly-Lys-Ser-Thr-Lys-Val-Pro-Ala-Ala-Tyr-Ala-Ala-Gln-Gly-
 35                  40                  45
Tyr-Lys-Val-Leu-Val-Leu-Asn-Pro-Ser-Val-Ala-Ala-Thr-Leu-Gly-
 50                  55                  60
Phe-Gly-Ala-Tyr-Met-Ser-Lys-Ala-His-Gly-Ile-Asp-Pro-Asn-Ile-
 65                  70                  75
Arg-Thr-Gly-Val-Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-
 80                  85                  90
Ser-Thr-Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-
 95                 100                 105
Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser
110                 115
```

SEQ ID No.: 274A
Sequence Type: AA
Sequence Length: 37 AA

```
Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-Ala-Arg-Leu-Val-Val-
  5                  10                  15
Leu-Ala-Thr-Ala-Thr-Pro-Pro-Gly-Ser-Val-Thr-Val-Pro-His-Pro-
 20                  25                  30
Asn-Ile-Glu-Glu-Val-Ala-Leu
 35
```

SEQ ID No.: 274B
Sequence Type: AA
Sequence Length: 64AA

```
Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-
 5                  10                  15
His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-
            20                  25                  30
Asp-Gln-Ala-Glu-Thr-Ala-Gly-Ala-Arg-Leu-Val-Val-Leu-Ala-Thr-
        35                  40                  45
Ala-Thr-Pro-Pro-Gly-Ser-Val-Thr-Val-Pro-His-Pro-Asn-Ile-Glu-
    50                  55                  60
Glu-Val-Ala-Leu
```

SEQ ID No.: 274C
Sequence Type: AA
Sequence Length: 97 AA

```
Ala-His-Gly-Ile-Asp-Pro-Asn-Ile-Arg-Thr-Gly-Val-Arg-Thr-Ile-
 5                  10                  15
Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-
            20                  25                  30
Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-
        35                  40                  45
Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-
    50                  55                  60
Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-Ala-Arg-Leu-Val-Val-
65                  70                  75
Leu-Ala-Thr-Ala-Thr-Pro-Pro-Gly-Ser-Val-Thr-Val-Pro-His-Pro-
            80                  85                  90
Asn-Ile-Glu-Glu-Val-Ala-Leu
        95
```

SEQ ID No.: **274D**
Sequence Type: AA
Sequence Length: 120 AA

```
Gly-Tyr-Lys-Val-Leu-Val-Leu-Asn-Pro-Ser-Val-Ala-Ala-Thr-Leu-
  5                  10                  15
Gly-Phe-Gly-Ala-Tyr-Met-Ser-Lys-Ala-His-Gly-Ile-Asp-Pro-Asn-
 20                  25                  30
Ile-Arg-Thr-Gly-Val-Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-
 35                  40                  45
Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-
 50                  55                  60
Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-
 65                  70                  75
Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-
 80                  85                  90
Thr-Ala-Gly-Ala-Arg-Leu-Val-Val-Leu-Ala-Thr-Ala-Thr-Pro-Pro-
 95                 100                 105
Gly-Ser-Val-Thr-Val-Pro-His-Pro-Asn-Ile-Glu-Glu-Val-Ala-Leu
110                 115                 120
```

SEQ ID No.: **262A**
Sequence Type: AA
Sequence Length: 29 AA

```
Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-
  5                  10                  15
Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr
 20                  25
```

SEQ ID No.: **262B**
Sequence Type: AA
Sequence Length: 39 AA

```
Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-
                    5                  10                  15
Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-
                20                  25                  30
Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr
                35
```

SEQ ID No.: **262C**
Sequence Type: AA
Sequence Length: 49 AA

```
Pro-Asn-Ile-Arg-Thr-Gly-Val-Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-
                    5                  10                  15
Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-
                20                  25                  30
Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-
                35                  40                  45
Thr-Asp-Ala-Thr
```

SEQ ID No.: **262D**
Sequence Type: AA
Sequence Length: 59 AA

```
Ala-Tyr-Met-Ser-Lys-Ala-His-Gly-Ile-Asp-Pro-Asn-Ile-Arg-Thr-
                    5                  10                  15
Gly-Val-Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-
                20                  25                  30
Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-
                35                  40                  45
Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr
                50                  55
```

SEQ ID No.: 262E
Sequence Type: AA
Sequence Length: 68 AA

```
Ser-Val-Ala-Ala-Thr-Leu-Gly-Phe-Gly-Ala-Tyr-Met-Ser-Lys-Ala-
                    5                  10
His-Gly-Ile-Asp-Pro-Asn-Ile-Arg-Thr-Gly-Val-Arg-Thr-Ile-Thr-
                20                  25                  30
Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-Ala-
                35                  40                  45
Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-
                50                  55                  60
Glu-Cys-His-Ser-Thr-Asp-Ala-Thr
                65
```

SEQ ID No.: 262F
Sequence Type: AA
Sequence Length: 77 AA

```
Gly-Tyr-Lys-Val-Leu-Val-Leu-Asn-Pro-Ser-Val-Ala-Ala-Thr-Leu-
                    5                  10                  15
Gly-Phe-Gly-Ala-Tyr-Met-Ser-Lys-Ala-His-Gly-Ile-Asp-Pro-Asn-
                20                  25                  30
Ile-Arg-Thr-Gly-Val-Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-
                35                  40                  45
Tyr-Ser-Thr-Tyr-Gly-Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-
                50                  55                  60
Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-
                65                  70                  75
Ala-Thr
```

SEQ ID No.: **261A**
Sequence Type: AA
Sequence Length: 30 AA

```
Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-
  5                  10                  15
Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-Glu-Leu
 20                  25                  30
```

SEQ ID No.: **261B**
Sequence Type: AA
Sequence Length: 40 AA

```
Glu-Val-Ala-Leu-Ser-Thr-Thr-Gly-Glu-Ile-Pro-Phe-Tyr-Gly-Lys-
  5                  10                  15
Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-
 20                  25                  30
Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-Glu-Leu
 35                  40
```

SEQ ID No.: **261C**
Sequence Type: AA
Sequence Length: 50 AA

```
Ser-Val-Thr-Val-Pro-His-Pro-Asn-Ile-Glu-Glu-Val-Ala-Leu-Ser-
  5                  10                  15
Thr-Thr-Gly-Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-
 20                  25                  30
Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-
 35                  40                  45
Lys-Cys-Asp-Glu-Leu
 50
```

SEQ ID No.: 261D
Sequence Type: AA
Sequence Length: 73 AA

```
Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-Ala-Arg-Leu-Val-Val-
 5                  10                  15
Leu-Ala-Thr-Ala-Thr-Pro-Pro-Gly-Ser-Val-Thr-Val-Pro-His-Pro-
            20                  25                  30
Asn-Ile-Glu-Glu-Val-Ala-Leu-Ser-Thr-Thr-Gly-Glu-Ile-Pro-Phe-
        35                  40                  45
Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-
    50                  55                  60
Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-Glu-Leu
65                  70
```

SEQ ID No.: 261E
Sequence Type: AA
Sequence Length: 97 AA

```
Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-
 5                  10                  15
Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-
            20                  25                  30
Glu-Thr-Ala-Gly-Ala-Arg-Leu-Val-Val-Leu-Ala-Thr-Ala-Thr-Pro-
        35                  40                  45
Pro-Gly-Ser-Val-Thr-Val-Pro-His-Pro-Asn-Ile-Glu-Glu-Val-Ala-
    50                  55                  60
Leu-Ser-Thr-Thr-Gly-Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-
65                  70                  75
Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-
            80                  85                  90
Lys-Lys-Lys-Cys-Asp-Glu-Leu
        95
```

SEQ ID No.: **261F**
Sequence Type: AA
Sequence Length: 121 AA

```
Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-
 5                  10                  15
Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-
            20                  25                  30
Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-
        35                  40                  45
Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-Ala-Arg-
    50                  55                  60
Leu-Val-Val-Leu-Ala-Thr-Ala-Thr-Pro-Pro-Gly-Ser-Val-Thr-Val-
65                  70                  75
Pro-His-Pro-Asn-Ile-Glu-Glu-Val-Ala-Leu-Ser-Thr-Thr-Gly-Glu-
            80                  85                  90
Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-
        95                  100                 105
Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-Glu-
    110                 115                 120
Leu
```

SEQ ID No.: **279A (Pep 3)**
Sequence Type: AA
Sequence Length: 37 AA

```
Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Cys-
 5                  10                  15
His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-
            20                  25                  30
Asp-Gln-Ala-Glu-Thr-Ala-Gly
        35
```

SEQ ID No.: 279B
Sequence Type: AA
Sequence Length: 42 AA

```
Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-
 5                  10                  15
Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-
            20                  25                  30
Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly
        35                  40
```

SEQ ID No.: 279E
Sequence Type: AA
Sequence Length: 58 AA

```
Arg-Thr-Ile-Thr-Thr-Gly-Ser-Pro-Ile-Thr-Tyr-Ser-Thr-Tyr-Gly-
 5                  10                  15
Lys-Phe-Leu-Ala-Asp-Gly-Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-
            20                  25                  30
Ile-Ile-Cys-Asp-Glu-Cys-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-
        35                  40                  45
Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly
    50                  55
```

SEQ ID No.: 255A
Sequence Type: AA
Sequence Length: 35 AA

```
Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-
 5                  10                  15
Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-
            20                  25                  30
Asn-Val-Ser-Arg-Cys
        35
```

SEQ ID No.: 255B
Sequence Type: AA
Sequence Length: 45 AA

```
Val-Arg-Asn-Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-
                    5                  10                  15
Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-
                20                  25                  30
Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-
                35                  40                  45
```

SEQ ID No.: 255C (Pep 7)
Sequence Type: AA
Sequence Length: 55 AA

```
Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-Ser-Thr-
                    5                  10                  15
Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-
                20                  25                  30
Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-
                35                  40                  45
Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys
                50                  55
```

SEQ ID No.: 244A
Sequence Type: AA
Sequence Length: 35 AA

```
Cys-Trp-Val-Ala-Met-Thr-Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-
                    5                  10                  15
Leu-Pro-Ala-Thr-Gln-Leu-Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-
                20                  25                  30
Ser-Ala-Thr-Leu-Cys
                35
```

SEQ ID No.: 244B
Sequence Type: AA
Sequence Length: 44 AA

```
Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-
                    5                  10                  15
Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-
                20                  25                  30
Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys
                35                  40
```

SEQ ID No.: 254A
Sequence Type: AA
Sequence Length: 30 AA

```
Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-
                    5                  10                  15
His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala
                20                  25                  30
```

SEQ ID No.: 254B (Pep 8)
Sequence Type: AA
Sequence Length: 40 AA

```
Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-
                    5                  10                  15
Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-
                20                  25                  30
Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala
                35                  40
```

SEQ ID No.: 254C
Sequence Type: AA
Sequence Length: 50 AA

```
Cys-Gly-Ser-Val-Phe-Leu-Ile-Gly-Gln-Leu-Phe-Thr-Phe-Ser-Pro-
 5                  10                  15
Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-
            20                  25                  30
Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-
        35                  40                  45
Trp-Ser-Pro-Thr-Ala
    50
```

SEQ ID No.: 248A
Sequence Type: AA
Sequence Length: 25 AA

```
Asp-Met-Ile-Ala-Gly-Ala-His-Trp-Gly-Val-Leu-Ala-Gly-Ile-Ala-
 5                  10                  15
Tyr-Phe-Ser-Met-Val-Gly-Asn-Trp-Ala-Lys
            20                  25
```

SEQ ID No.: 248B
Sequence Type: AA
Sequence Length: 35 AA

```
Gln-Leu-Leu-Arg-Ile-Pro-Gln-Ala-Ile-Leu-Asp-Met-Ile-Ala-Gly-
 5                  10                  15
Ala-His-Trp-Gly-Val-Leu-Ala-Gly-Ile-Ala-Tyr-Phe-Ser-Met-Val-
            20                  25                  30
Gly-Asn-Trp-Ala-Lys
        35
```

SEQ ID No.: **248C**
Sequence Type: AA
Sequence Length: 40 AA

```
Ala-Leu-Val-Met-Ala-Gln-Leu-Leu-Arg-Ile-Pro-Gln-Ala-Ile-Leu-
                5                  10                  15
Asp-Met-IleAla-Gly-Ala-His-Trp-Gly-Val-Leu-Ala-Gly-Ile-Ala-
            20                  25                  30
Tyr-Phe-Ser-Met-Val-Gly-Asn-Trp-Ala-Lys
            35                  40
```

SEQ ID No.: **247A**
Sequence Type: AA
Sequence Length: 25 AA

```
Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-
                5                  10                  15
Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn
            20                  25
```

SEQ ID No.: **247B (Pep 9)**
Sequence Type: AA
Sequence Length: 35 AA

```
Val-Asp-Ala-Glu-Thr-Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-
                5                  10                  15
Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-
            20                  25                  30
Ile-Gln-Leu-Ile-Asn
            35
```

SEQ ID No.: 247C
Sequence Type: AA
Sequence Length: 45AA

```
Val-Leu-Val-Val-Leu-Leu-Leu-Phe-Ala-Gly-Val-Asp-Ala-Glu-Thr-
 5                  10                  15
Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-
 20                  25                  30
Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn
 35                  40                  45
```

SEQ ID No.: 247D
Sequence Type: AA
Sequence Length: 55 AA

```
Tyr-Phe-Ser-Met-Val-Gly-Asn-Trp-Ala-Lys-Val-Leu-Val-Val-Leu-
 5                  10                  15
Leu-Leu-Phe-Ala-Gly-Val-Asp-Ala-Glu-Thr-Ile-Val-Ser-Gly-Gly-
 20                  25                  30
Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-
 35                  40                  45
Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn
 50                  55
```

SEQ ID No.: 247E
Sequence Type: AA
Sequence Length: 60 AA

```
Leu-Ala-Gly-Ile-Ala-Tyr-Phe-Ser-Met-Val-Gly-Asn-Trp-Ala-Lys-
 5                  10                  15
Val-Leu-Val-Val-Leu-Leu-Leu-Phe-Ala-Gly-Val-Asp-Ala-Glu-Thr-
 20                  25                  30
Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-
 35                  40                  45
Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn
 50                  55                  60
```

SEQ ID No.: 246A
Sequence Type: AA
Sequence Length: 25 AA

Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Gln-His-Lys-Phe-Asn-Ser-
5 10 15
Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys
20 25

SEQ ID No.: 246B
Sequence Type: AA
Sequence Length: 31 AA

Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-
5 10 15
Gln-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-
20 25 30
Cys

SEQ ID No.: 246C
Sequence Type: AA
Sequence Length: 38 AA

Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-
5 10 15
Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Gln-His-Lys-Phe-Asn-Ser-Ser-Gly-
20 25 30
Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys
35

SEQ ID No.: **246D (Pep 10)**
Sequence Type: AA
Sequence Length: 40 AA

```
Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-
 5                  10                  15
Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Gln-His-Lys-Phe-Asn-Ser-
            20                  25                  30
Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys
            35                  40
```

SEQ ID No.: **246E**
Sequence Type: AA
Sequence Length: 52 AA

```
Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn-Thr-Asn-Gly-Ser-Trp-His-Ile-
 5                  10                  15
Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-Trp-
            20                  25                  30
Leu-Ala-Gly-Leu-Ile-Tyr-Gln-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-
            35                  40                  45
Pro-Glu-Arg-Leu-Ala-Ser-Cys
            50
```

SEQ ID No.: **314A (Pep 16)**

Sequence Length: 30 AA

```
Gly-Ser-Trp-Leu-Arg-Asp-Ile-Trp-Asp-Trp-Ile-Cys-Glu-Val-Leu-
 5                  10                  15
Ser-Asp-Phe-Lys-Thr-Trp-Leu-Lys-Ala-Lys-Leu-Met-Pro-Gln-Leu
            20                  25                  30
```

SEQ ID No.: **314B**
Sequence Type: AA
Sequence Length: 38 AA

Ser-Glu-Cys-Thr-Thr-Pro-Cys-Ser-Gly-Ser-Trp-Leu-Arg-Asp-Ile-
5 10 15
Trp-Asp-Trp-Ile-Cys-Glu-Val-Leu-Ser-Asp-Phe-Lys-Thr-Trp-Leu-
20 25 30
Lys-Ala-Lys-Leu-Met-Pro-Gln-Leu
35

SEQ ID No.: **314C**
Sequence Type: AA
Sequence Length: 47 AA

Leu-Arg-Arg-Leu-His-Gln-Trp-Ile-Ser-Ser-Glu-Cys-Thr-Thr-Pro-
5 10 15
Cys-Ser-Gly-Ser-Trp-Leu-Arg-Asp-Ile-Trp-Asp-Trp-Ile-Cys-Glu-
20 25 30
Val-Leu-Ser-Asp-Phe-Lys-Thr-Trp-Leu-Lys-Ala-Lys-Leu-Met-Pro-
35 40 45
Gln-Leu

SEQ ID No.: **312A**
Sequence Type: AA
Sequence Length: 22 AA

Asp-Phe-His-Tyr-Val-Thr-Gly-Met-Thr-Thr-Asp-Asn-Leu-Lys-Cys-
5 10 15
Pro-Cys-Gln-Val-Pro-Ser-Pro
20

SEQ ID No.: **312B (Pep 15)**
Sequence Type: AA
Sequence Length: 32 AA

Glu-Glu-Tyr-Val-Glu-Ile-Arg-Gln-Val-Gly-Asp-Phe-His-Tyr-Val-
5 10 15
Thr-Gly-Met-Thr-Thr-Asp-Asn-Leu-Lys-Cys-Pro-Cys-Gln-Val-Pro-
20 25 30
Ser-Pro

SEQ ID No.: **312C**
Sequence Type: AA
Sequence Length: 38 AA

Leu-Trp-Arg-Val-Ser-Ala-Glu-Glu-Tyr-Val-Glu-Ile-Arg-Gln-Val-
5 10 15
Gly-Asp-Phe-His-Tyr-Val-Thr-Gly-Met-Thr-Thr-Asp-Asn-Leu-Lys-
20 25 30
Cys-Pro-Cys-Gln-Val-Pro-Ser-Pro
35

SEQ ID No.: **311A (Pep 14)**
Sequence Type: AA
Sequence Length: 31 AA

Cys-Lys-Pro-Leu-Leu-Arg-Glu-Glu-Val-Ser-Phe-Arg-Val-Gly-Leu-
5 10 15
His-Glu-Tyr-Pro-Val-Gly-Ser-Gln-Leu-Pro-Cys-Glu-Pro-Glu-Pro-
20 25 30
Asp

SEQ ID No.: **311B**
Sequence Type: AA
Sequence Length: 42 AA

```
Asp-Gly-Val-Arg-Leu-His-Arg-Phe-Ala-Pro-Pro-Cys-Lys-Pro-Leu-
                  5                  10                  15
Leu-Arg-Glu-Glu-Val-Ser-Phe-Arg-Val-Gly-Leu-His-Glu-Tyr-Pro-
                 20                  25                  30
Val-Gly-Ser-Gln-Leu-Pro-Cys-Glu-Pro-Glu-Pro-Asp
                 35                  40
```

SEQ ID No.: **311C**
Sequence Type: AA
Sequence Length: 54 AA

```
Cys-Gln-Val-Pro-Ser-Pro-Glu-Phe-Phe-Thr-Glu-Leu-Asp-Gly-Val-
                  5                  10                  15
Arg-Leu-His-Arg-Phe-Ala-Pro-Pro-Cys-Lys-Pro-Leu-Leu-Arg-Glu-
                 20                  25                  30
Glu-Val-Ser-Phe-Arg-Val-Gly-Leu-His-Glu-Tyr-Pro-Val-Gly-Ser-
                 35                  40                  45
Gln-Leu-Pro-Cys-Glu-Pro-Glu-Pro-Asp
                 50
```

SEQ ID No.: **260A**
Sequence Type: AA
Sequence Length: 23 AA

```
Ser-Ser-Ser-Ala-Ser-Gln-Leu-Ser-Ala-Pro-Ser-Leu-Lys-Ala-Thr-
                  5                  10                  15
Cys-Thr-Ala-Asn-His-Asp-Ser-Pro
                 20
```

SEQ ID No.: **260B**
Sequence Type: AA
Sequence Length: 35 AA

```
Arg-Arg-Leu-Ala-Arg-Gly-Ser-Pro-Pro-Ser-Val-Ala-Ser-Ser-Ser-
  5                  10                  15
Ala-Ser-Gln-Leu-Ser-Ala-Pro-Ser-Leu-Lys-Ala-Thr-Cys-Thr-Ala-
 20                  25                  30
Asn-His-Asp-Ser-Pro
 35
```

SEQ ID No.: **260C (Pep 4)**
Sequence Type: AA
Sequence Length: 46 AA

```
Asp-Pro-Ser-His-Ile-Thr-Ala-Glu-Ala-Ala-Gly-Arg-Arg-Leu-Ala-
  5                  10                  15
Arg-Gly-Ser-Pro-Pro-Ser-Val-Ala-Ser-Ser-Ala-Ser-Gln-Leu-
 20                  25                  30
Ser-Ala-Pro-Ser-Leu-Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-
 35                  40                  45
Pro
```

SEQ ID No.: **259B**
Sequence Type: AA
Sequence Length: 35 AA

```
Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-
  5                  10                  15
Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-
 20                  25                  30
Glu-Glu-Asp-Glu-Arg
 35
```

SEQ ID No.: 259C (Pep 5)
Sequence Type: AA
Sequence Length: 44 AA

```
Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-
                  5                  10                  15
Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-
              20                  25                  30
Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg
              35                  40
```

SEQ ID No.: 259D
Sequence Type: AA
Sequence Length: 50 AA

```
Ala-Asn-His-Asp-Ser-Pro-Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-
                  5                  10                  15
Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-
              20                  25                  30
Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-
              35                  40                  45
Glu-Glu-Asp-Glu-Arg
              50
```

SEQ ID No.: 259E (Pep 12)
Sequence Type: AA
Sequence Length: 55 AA

```
Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-Asp-Ala-Glu-Leu-
                  5                  10                  15
Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-
              20                  25                  30
Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-
              35                  40                  45
Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg
              50                  55
```

SEQ ID No.: 310A
Sequence Type: AA
Sequence Length: 26 AA

```
Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Ile-Ser-
 5                  10                  15
Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg
            20                  25
```

SEQ ID No.: 310B
Sequence Type: AA
Sequence Length: 35 AA

```
Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-
 5                  10                  15
Ala-Glu-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-
            20                  25                  30
Arg-Lys-Ser-Arg-Arg
            35
```

SEQ ID No.: 310C (Pep 13)
Sequence Type: AA
Sequence Length: 47 AA

```
Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-
 5                  10                  15
Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-
            20                  25                  30
Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-
            35                  40                  45
Arg-Arg
```

SEQ ID No.: 309A
Sequence Type: AA
Sequence Lenghh: 27 AA

Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-
5 10 15
Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr
20 25

SEQ ID No.: 309B
Sequence Type: AA
Sequence Lentgh: 35 AA

Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-
5 10 15
Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-
20 25 30
Arg-Lys-Lys-Arg-Thr
35

SEQ ID No.: 309C
Sequence Type: AA
Sequence Length: 44 AA

Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-
5 10 15
Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-
20 25 30
Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr
35 40

SEQ ID No.: 309D (Pep 11)
Sequence Type: AA
Sequence Length: 60 AA

```
Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-
 5                  10                  15
Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-
 20                  25                  30
Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-
 35                  40                  45
Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr
 50                  55                  60
```

SEQ ID No.: 309E
Sequence Type: AA
Sequence Length: 72 AA

```
Ala-Glu-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-
 5                  10                  15
Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-
 20                  25                  30
Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-
 35                  40                  45
Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-
 50                  55                  60
Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr
 65                  70
```

SEQ ID No.: Pep 6
Sequence Type: AA
Sequence Length: 37 AA

```
Asp-Pro-Gln-Ala-Arg-Val-Ala-Ile-Lys-Ser-Leu-Thr-Glu-Arg-Leu-
 5                  10                  15
Thr-Val-Gly-Gly-Pro-Leu-Thr-Asn-Ser-Arg-Gly-Glu-Asn-Cys-Gly-
 20                  25                  30
Tyr-Arg-Arg-Cys-Arg-Ala-Ser
 35
```

SEQ ID No.: **Pep 17**
Sequence Type: AA
Sequence Length: 45 AA

```
Gly-Pro-Ala-Asp-Gly-Met-Val-Ser-Lys-Gly-Trp-Arg-Leu-Leu-Ala-
 5                  10                  15
Pro-Ile-Thr-Ala-Tyr-Ala-Gln-Gln-Thr-Arg-Gly-Leu-Leu-Gly-Cys-
            20                  25                  30
Ile-Ile-Thr-Ser-Leu-Thr-Gly-Arg-Asp-Lys-Asn-Gln-Val-Glu-Gly
        35                  40                  45
```

SEQ ID No.: **Pep 18**
Sequence Type: AA
Sequence Length: 39 AA

```
Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-
 5                  10                  15
Gly-Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-
            20                  25                  30
Glu-Leu-Ala-Ala-Lys-Leu-Val-Ala-Leu
        35
```

SEQ ID No.: **Pep 19**
Sequence Type: AA
Sequence Length: 44 AA

```
Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-
 5                  10                  15
Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-
            20                  25                  30
Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys
        35                  40
```

SEQ ID No.: VIIIE
Sequence Type: AA
Sequence Length: 61 AA

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-
5 10 15
Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-
20 25 30
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-
35 40 45
Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-
50 55 60
Arg

SEQ ID No.: IIH
Sequence Type: AA
Sequence Length: 47 AA

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-
5 10 15
Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-
20 25 30
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-
35 40 45
Gly-Leu

SEQ ID No.: V
Sequence Type: AA
Sequence Length: 40 AA

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-
5 10 15
Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-
20 25 30
Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe
35 40

SEQ ID No.: **Pep XX**
Sequence Type: AA
$[Peptide]_{16}$ Lys_8 Lys_4 Lys_2 Lys-Y
decahexyl peptide

SEQ ID No.: **Pep XXI**
Sequence Type: AA
$[Peptide]_8$ Lys_4 Lys_2 Lys-Y
octameric peptide

SEQ ID No.: **Pep XXII**
Sequence Type: AA
$[Peptide]_4$ Lys_2 Lys-Y
tetrameric peptide

SEQ ID No.: **Pep XXIII**
Sequence Type: AA
$[Peptide]_2$ Lys-Y
dimeric peptide

wherein the peptide in Pep XX, Pep XXI, Pep XXII and Pep XIII is selected from the group consisting of (a) to (j):

(a) Cys-Leu-Thr-Ile-Pro-Ala-Ser-Ala-Tyr-Glu-Val-Arg-Asn-Val-Ser-
5 10 15
Gly-Ile-Tyr-His-Val-Thr-Asn-Asp-Cys-Ser-Asn-Ser-Ser-Ile-Val-
20 25 30
Tyr-Glu-Ala-Ala-Asp-Val-Ile-Met-His-Ala-Pro-Gly-Cys-Val-Pro-
35 40 45
Cys-Val-Arg-Glu-Asn-Asn-Ser-Ser-Arg-Cys
50 55

(b) Cys-Ile-Thr-Thr-Pro-Val-Ser-Ala-Ala-Glu-Val-Lys-Asn-Ile-Ser-
5 10 15
Thr-Gly-Tyr-Met-Val-Thr-Asn-Asp-Cys-Thr-Asn-Asp-Ser-Ile-Thr-
20 25 30
Trp-Gln-Leu-Gln-Ala-Ala-Val-Leu-His-Val-Pro-Gly-Cys-Val-Pro-
35 40 45
Cys-Glu-Lys-Val-Gly-Asn-Thr-Ser-Arg-Cys
50 55

(c) Cys-Val-Thr-Val-Pro-Val-Ser-Ala-Val-Glu-Val-Arg-Asn-Ile-Ser-
5 10 15
Ser-Ser-Tyr-Tyr-Ala-Thr-Asn-Asp-Cys-Ser-Asn-Asn-Ser-Ile-Thr-
20 25 30
Trp-Gln-Leu-Thr-Asn-Ala-Val-Leu-His-Leu-Pro-Gly-Cys-Val-Pro-
35 40 45
Cys-Glu-Asn-Asp-Asn-Gly-Thr-Leu-Arg-Cys
50 55

(d) Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Glu-Thr-Val-Gln-Asp-Cys-Asn-
5 10 15
Cys-Ser-Ile-Tyr-Pro-Gly-His-Val-Ser-Gly-His-Arg-Met-Ala-Trp-
20 25 30
Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala
35 40

(e) Phe-Ile-Val-Ser-Pro-Gln-His-His-His-Phe-Val-Gln-Asp-Cys-Asn-
5 10 15
Cys-Ser-Ile-Tyr-Pro-Gly-Thr-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-
20 25 30
Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala
35 40

(f) Phe-Ile-Ile-Ser-Pro-Glu-Arg-Asn-Phe-Thr-Gln-Glu-Cys-Asn-Cys-
5 10 15
Ser-Ile-Tyr-Gln-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-
20 25 30
Met-Met-Leu-Asn-Trp-Ser-Pro-Thr-Leu
35

(g) Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-
5 10 15
Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-
20 25 30
Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys
35 40

(h) Cys-Val-Arg-Glu-Asn-Asn-Ser-Ser-Arg-Cys-Trp-Val-Ala-Leu-Thr-
5 10 15
Pro-Thr-Leu-Ala-Ala-Arg-Asn-Ala-Ser-Val-Pro-Thr-Thr-Thr-Leu-
20 25 30
Arg-Arg-His-Val-Asp-Leu-Leu-Val-Gly-Thr-Ala-Ala-Phe-Cys
35 40

(i) Cys-Glu-Lys-Val-Gly-Asn-Thr-Ser-Arg-Cys-Trp-Ile-Pro-Val-Ser-
5 10 15
Pro-Asn-Val-Ala-Val-Gln-Gln-Pro-Gly-Ala-Leu-Thr-Gln-Gly-Leu-
20 25 30
Arg-Thr-His-Ile-Asp-Met-Val-Val-Met-Ser-Ala-Thr-Leu-Cys
35 40

(j) Cys-Glu-Asn-Asp-Asn-Gly-Thr-Leu-Arg-Cys-Trp-Ile-Gln-Val-Thr-
5 10 15
Pro-Asn-Val-Ala-Val-Lys-His-Arg-Gly-Ala-Leu-Thr-His-Asn-Leu-
20 25 30
Arg-Thr-His-Val-Asp-Met-Ile-Val-Met-Ala-Ala-Thr-Val-Cys
35 40

Claims

1. A peptide composition comprising a peptide having an amino acid sequence selected from the group consisting of:

(a) Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-Val-Tyr-Cys-X;

Pep1

(b) Pro-Pro-Leu-Gly-Asn-Trp-Phe-Gly-Cys-Thr-Trp-Met-Asn-Ser-Thr-Gly-Phe-Thr-Lys-Val-Cys-Gly-Ala-Pro-Pro-Cys-X;

Pep2

(c) Gly-Cys-Ser-Gly-Gly-Ala-Tyr-Asp-Ile-Ile-Ile-Cys-Asp-Glu-Leu-His-Ser-Thr-Asp-Ala-Thr-Ser-Ile-Leu-Gly-Ile-Gly-Thr-Val-Leu-Asp-Gln-Ala-Glu-Thr-Ala-Gly-X;

Pep3

(d) Asp-Pro-Ser-His-Ile-Thr-Ala-Glu-Ala-Ala-Gly-Arg-Arg-Leu-Ala-Arg-Gly-Ser-Pro-Pro-Ser-Val-Ala-Ser-Ser-Ser-Ala-Ser-Gln-Leu-Ser-Ala-Pro-Ser-Leu-Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-X;

Pep4

(e) Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X;

Pep5

(f) Asp-Pro-Gln-Ala-Arg-Val-Ala-Ile-Lys-Ser-Leu-Thr-Glu-Arg-Leu-Thr-Val-Gly-Gly-Pro-Leu-Thr-Asn-Ser-Arg-Gly-Glu-Asn-Cys-Gly-Tyr-Arg-Arg-Cys-Arg-Ala-Ser-X;

Pep6

(g) Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-X;

Pep7

(h) Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala-X;

Pep8

(i) Val-Asp-Ala-Glu-Thr-Ile-Val-Ser-Gly-Gly-Gln-Ala-Ala-Arg-Ala-Met-Ser-Gly-Leu-Val-Ser-Leu-Phe-Thr-Pro-Gly-Ala-Lys-Gln-Asn-Ile-Gln-Leu-Ile-Asn-X;

Pep9

(j) Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Glu-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys-X;

Pep10

(k) Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr-X;

Pep11

(l) Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X;

Pep12

(m) Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-X;

Pep13

(n) Cys-Lys-Pro-Leu-Leu-Arg-Glu-Glu-Val-Ser-Phe-Arg-Val-Gly-Leu-His-Glu-Tyr-Pro-Val-Gly-Ser-Gln-Leu-Pro-Cys-Glu-Pro-Glu-Pro-Asp-X;

Pep14

(o) Glu-Glu-Tyr-Val-Glu-Ile-Arg-Gln-Val-Gly-Asp-Phe-His-Tyr-Val-Thr-Gly-Met-Thr-Thr-Asp-Asn-Leu-Lys-Cys-Pro-Cys-Gln-Val-Pro-Ser-Pro-X;

Pep15

(p) Gly-Ser-Trp-Leu-Arg-Asp-Ile-Trp-Asp-Trp-Ile-Cys-Glu-Val-Leu-Ser-Asp-Phe-Lys-Thr-Trp-Leu-Lys-Ala-Lys-Leu-Met-Pro-Gln-Leu-X;

Pep16

(q) Gly-Pro-Ala-Asp-Gly-Met-Val-Ser-Lys-Gly-Trp-Arg-Leu-Leu-Ala-Pro-Ile-Thr-Ala-Tyr-Ala-Gln-Gln-Thr-Arg-Gly-Leu-Leu-Gly-Cys-Ile-Ile-Thr-Ser-Leu-Thr-Gly-Arg-Asp-Lys-Asn-Gln-Val-Glu-Gly-X;

Pep17

(r) Glu-Ile-Pro-Phe-Tyr-Gly-Lys-Ala-Ile-Pro-Leu-Glu-Val-Ile-Lys-Gly-Gly-Arg-His-Leu-Ile-Phe-Cys-His-Ser-Lys-Lys-Lys-Cys-Asp-Glu-Leu-Ala-Ala-Lys-Leu-Val-Ala-Leu-X;

Pep18

(s) Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys-X;

Pep19

wherein X is -OH or -NH_2, and analogues, segments, mixtures, conjugates and polymers thereof.

2. A peptide composition according to claim 1 wherein the peptide comprises:

Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-X;

pep7

wherein X is -OH or -NH_2, and analogues, segments, conjugates and polymers thereof.

3. A peptide composition according to Claim 1 wherein the peptide comprises:

Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala-X;

pep8

wherein X is -OH or -NH_2, and analogues, segments, conjugates and polymers thereof.

4. A peptide composition according to Claim 1 wherein the peptide comprises:

Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-Leu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Glu-His-Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-Cys-X;

pep10

wherein X is -OH or -NH_2, and analogues, segments, conjugates and polymers thereof.

5. A peptide composition according to Claim 1 wherein the peptide comprises:

Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-
Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-
Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-
His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-
Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr-X;

pep11

wherein X is -OH or -NH_2, and analogues, segments, conjugates and polymers thereof.

6. A peptide composition comprising a mixture of Peptides VIIIE and pep11 wherein Peptide VIIIE is:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-
Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-
Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-
Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-
Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

(VIIIE)

and pep11 is:

Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-
Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-
Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-
His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-
Pro-Pro-Pro-Arg-Lys-Lys-Arg-Thr-X;

Pep11

wherein X is -OH or NH_2, and analogues thereof.

7. A peptide composition according to claim 6 further comprising Peptide IIH having an amino acid sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-
Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-
Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-
Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(IIH)

wherein X -OH or -NH_2 and analogues thereof.

8. A peptide composition according to Claim 6 further comprising pep8 having an amino acid sequence:

Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala-X;

Pep8

wherein X is -OH or -NH_2, and analogues thereof.

9. A peptide composition according to Claim 6 further comprising pep12 having an amino acid sequence:

Lys-Ala-Thr-Cys-Thr-Ala-Asn-His-Asp-Ser-Pro-Asp-Ala-Glu-Leu-Ile-Glu-Ala-Asn-Leu-Leu-Trp-Arg-Gln-Glu-Met-Gly-Gly-Asn-Ile-Thr-Arg-Val-Glu-Ser-Glu-Asn-Lys-Val-Val-Ile-Leu-Asp-Ser-Phe-Asp-Pro-Leu-Val-Ala-Glu-Glu-Asp-Glu-Arg-X;

Pep12

wherein X is -OH or -NH_2 and analogues thereof.

10. A peptide composition comprising a mixture of Peptides VIIIE and pep8 wherein Peptide VIIIE is

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

(VIIIE)

and pep8 is:

Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-
Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-
Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-
Ala-X;

Pep8

and wherein X is -OH or -NH_2 and analogues thereof.

11. A peptide composition according to Claim 1 comprising a mixture of pep7 and pep8, wherein pep7 is:

Cys-Leu-Thr-Val-Pro-Ala-Ser-Ala-Tyr-Gln-Val-Arg-Asn-
Ser-Thr-Gly-Leu-Tyr-His-Val-Thr-Asn-Asp-Cys-Pro-Asn-
Ser-Ser-Ile-Val-Tyr-Glu-Ala-His-Asp-Ala-Ile-Leu-His-
Thr-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Gly-Asn-Val-
Ser-Arg-Cys-X;

Pep7

and pep8 is:

Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Trp-Thr-Thr-Gln-Gly-
Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Ile-Thr-Gly-His-
Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-
Ala-X;

Pep8

and wherein X is -OH or -NH_2, and analogues thereof.

12. A peptide composition according to Claim 1 comprising a mixture of pep1 and pep10, wherein pep1 is:

Gln-Gly-Trp-Gly-Pro-Ile-Ser-Tyr-Ala-Asn-Gly-Ser-Gly-
Pro-Asp-Gln-Arg-Pro-Tyr-Cys-Trp-His-Tyr-Pro-Pro-Lys-
Pro-Cys-Gly-Ile-Val-Pro-Ala-Lys-Ser-Val-Cys-Gly-Pro-
Val-Tyr-Cys-X;

Pep1

pep10 is:

Trp-His-Ile-Asn-Ser-Thr-Ala-Leu-Asn-Cys-Asn-Glu-Ser-
Leu-Asn-Thr-Gly-Trp-Leu-Ala-Gly-Leu-Ile-Tyr-Glu-His-
Lys-Phe-Asn-Ser-Ser-Gly-Cys-Pro-Glu-Arg-Leu-Ala-Ser-
Cys-X;

Pep10

and wherein X is -OH or -NH_2, and analogues thereof.

13. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of a peptide composition according to Claim 1 in an immunoassay procedure.

14. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of a peptide composition according to Claim 6 in an immunoassay procedure.

15. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of a peptide composition according to Claim 7 In an immunoassay procedure.

16. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of a peptide composition according to Claim 8 in an immunoassay procedure.

17. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of a peptide composition according to Claim 9 in an immunoassay procedure.

18. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using on effective amount of a peptide composition according to Claim 10 in an immunoassay procedure.

19. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of a peptide composition according to Claim 11 in an immunoassay procedure.

20. A method of detecting antibodies to HCV or diagnosis of HCV infection or NANBH by using an effective amount of peptide composition according to Claim 12 in an immunoassay procedure.

21. A peptide immunogen comprising a polymeric peptide selected from the group consisting of:
[Peptide]$_{16}$ Lys_8 Lys_4 Lys_2 Lys-Y
[Peptide]$_8$ Lys_4 Lys_2 Lys-Y
[Peptide]$_4$ Lys_2 Lys-Y
[Peptide]$_2$ Lys-Y
wherein Y is -OH_2, -NH_2 or amino acid with no side chain functional group and the peptide is selected from the group consisting of:

(a) Cys-Leu-Thr-Ile-Pro-Ala-Ser-Ala-Tyr—Glu-Val-Arg-Asn-Val-Ser-Gly-Ile-Tyr—His-Val-Thr-Asn-Asp-Cys-Ser-Asn-Ser-Ser-Ile-Val-Tyr—Glu-Ala-Ala-Asp-Val-Ile-Met-His-Ala-Pro-Gly-Cys-Val-Pro-Cys-Val-Arg-Glu-Asn-Asn-Ser-Ser-Arg-Cys;

(b) Cys-Ile-Thr-Thr-Pro-Val-Ser-Ala-Ala-Glu-Val-Lys-Asn-Ile-Ser-Thr-Gly-Tyr—Met-Val-Thr-Asn-Asp-Cys-Thr-Asn-Asp-Ser-Ile-Thr-Trp-Gln-Leu-Gln-Ala-Ala-Val-Leu-His-Val-Pro-Gly-Cys-Val-Pro-Cys-Glu-Lys-Val-Gly-Asn-Thr-Ser-Arg-Cys;

(c) Cys-Val-Thr-Val-Pro-Val-Ser-Ala-Val-Glu-Val-Arg-Asn-Ile-Ser-Ser-Ser-Tyr—Tyr—Ala-Thr-Asn-Asp-Cys-Ser-Asn-Asn-Ser-Ile-Thr-Trp-Gln-Leu-Thr-Asn-Ala-Val-Leu-His-Leu-Pro-Gly-Cys-Val-Pro-Cys-Glu-Asn-Asp-Asn-Gly-Thr-Leu-Arg-Cys;

(d) Phe-Thr-Phe-Ser-Pro-Arg-Arg-His-Glu-Thr-Val-Gln-Asp-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-His-Val-Ser-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala;

(e) Phe-Ile-Val-Ser-Pro-Gln-His-His-His-Phe-Val-Gln-Asp-Cys-Asn-Cys-Ser-Ile-Tyr-Pro-Gly-Thr-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Met-Asn-Trp-Ser-Pro-Thr-Ala;

(f) Phe-Ile-Ile-Ser-Pro-Glu-Arg-Asn-Phe-Thr-Gln-Glu-Cys-Asn-Cys-Ser-Ile-Tyr-Gln-Gly-His-Ile-Thr-Gly-His-Arg-Met-Ala-Trp-Asp-Met-Met-Leu-Asn-Trp-Ser-Pro-Thr-Leu;

(g) Cys-Val-Arg-Glu-Gly-Asn-Val-Ser-Arg-Cys-Trp-Val-Ala-Met-Thr-Pro-Thr-Val-Ala-Thr-Arg-Asp-Gly-Lys-Leu-Pro-Ala-Thr-Gln-Leu-Arg-Arg-His-Ile-Asp-Leu-Leu-Val-Gly-Ser-Ala-Thr-Leu-Cys;

(h) Cys-Val-Arg-Glu-Asn-Asn-Ser-Ser-Arg-Cys-Trp-Val-Ala-Leu-Thr-Pro-Thr-Leu-Ala-Ala-Arg-Asn-Ala-Ser-Val-Pro-Thr-Thr-Thr-Leu-Arg-Arg-His-Val-Asp-Leu-Leu-Val-Gly-Thr-Ala-Ala-Phe-Cys;

(i) Cys-Glu-Lys-Val-Gly-Asn-Thr-Ser-Arg-Cys-Trp-Ile-Pro-Val-Ser-Pro-Asn-Val-Ala-Val-Gln-Gln-Pro-Gly-Ala-Leu-Thr-Gln-Gly-Leu-Arg-Thr-His-Ile-Asp-Met-Val-Val-Met-Ser-Ala-Thr-Leu-Cys; and

(j) Cys-Glu-Asn-Asp-Asn-Gly-Thr-Leu-Arg-Cys-Trp-Ile-Gln-Val-Thr-Pro-Asn-Val-Ala-Val-Lys-His-Arg-Gly-Ala-Leu-Thr-His-Asn-Leu-Arg-Thr-His-Val-Asp-Met-Ile-Val-Met-Ala-Ala-Thr-Val-Cys;

and analogues thereof.

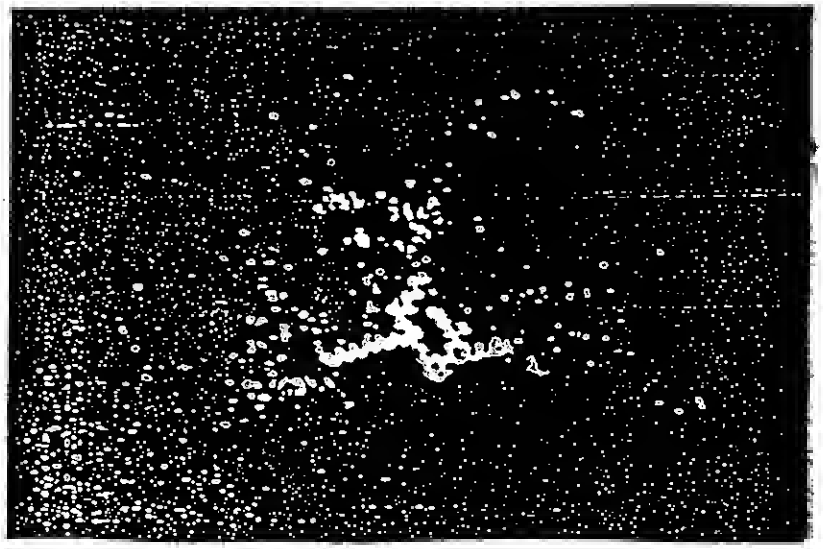


Fig. 1